UNIVERSIDADE ESTADUAL PAULISTA

"Julio de Mesquita Filho"

INSTITUTO DE BIOCIÊNCIAS DE BOTUCATU

Análise integrativa do transcriptoma e proteoma da próstata ventral da prole de ratos submetidos à restrição proteica materna

Ana Carolina Lima Camargo
Discente
Prof. Dr. Luis Antonio Justulin Junior
Orientador
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Coorientador

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Tese apresentada ao Instituto de Biociências, Campus de Botucatu, UNESP, para obtenção do título de Doutor no Programa de Pós-Graduação em Biologia Geral e Aplicada, Área de concentração *Biologia Celular e Estrutural e Funcional.*

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“Nothing in life is to be feared, it is only to be understood. Now is the time to understand more, so that we may fear less”

Marie Curie

Resumo

A exposição materna à dieta deficiente em proteína durante a gestação, está associada ao desenvolvimento de doenças na prole, em especial cardiovasculares, renais, metabólicas e até mesmo o câncer. Além disso, estudos do nosso grupo de pesquisa demonstram que a restrição proteica materna (RPM) impacta o desenvolvimento e aumenta a incidência de lesões prostáticas na prole de ratos velhos. Os mecanismos moleculares que desencadeiam estas alterações ainda são pouco conhecidos. Assim, o objetivo deste trabalho foi identificar o perfil de expressão global de RNAs mensageiros (transcriptoma) e de proteínas (proteoma) em amostras de próstata ventral de ratos jovens submetidos à RPM durante a gestação e lactação, e integrar vias moleculares envolvidas no desenvolvimento prostático em animais normais e restritos. Utilizamos ratos machos da linhagem *Sprague Dawley* no dia pós natal (DPN) 21 nascidos de mães alimentadas com ração padrão (17% de proteína) (grupo controle -CTR), ou de mães alimentadas com ração hipoproteica (6% de proteína) durante a gestação e lactação (Grupo restrito gestação e lactação (GLLP). Após este período, os animais foram eutanasiados com overdose de anestésico, pesados e a próstata ventral (PV) coletada. Observamos atraso nas etapas do desenvolvimento prostático (menor peso corpóreo e peso PV ao nascimento no DPN 1), acompanhado do desbalanço hormonal (aumento nos níveis de colesterol, testosterona e estrógeno no DPN 21). Análises de proteoma (LC-MS/MS) revelou alteração no perfil protêomico da glândula prostática desses animais, apresentando o enriquecimento de vias moleculares associadas ao perfil de perturbação estrogênica e câncer. Análise de transcriptoma (HigSeq-2500, Ilumina) revelou perfil gênico distinto entre os grupos CTR e GLLP, apresentando genes codificadores de proteínas envolvidos em vias de desenvolvimento, citocromo p450 e câncer. Ambos resultados foram comparados aos dados de transcriptoma e do proteoma para adenocarcionoma prostático (PRAD) no banco de dados *The Cancer Genome Atlas*, com o objetivo de investigar se a RPM é responsável por alterar a expressão gênica e proteica de alvos relacionados ao desenvolvimento de lesões prostáticas, dados associados aos animais no DPN 50 submetidos a RPM. Nossos resultados destacam que o papel da RPM, no desenvolvimento normal da próstata, alterando as vias moleculares que promovem a carcinogênese da próstata de crescimento lento com o envelhecimento. Além disso, a análise *in sílico* pode ser uma ferramenta útil para caracterizar potenciais biomarcadores e vias moleculares envolvidas na carcinogênese da próstata de roedores e humanos.

Palavras- chave: Próstata, DoHaD, Transcriptoma e Proteoma

Abstract

Maternal exposure to a protein-deficient diet during pregnancy is associated with the development of diseases in the offspring, especially cardiovascular, renal, metabolic and even cancer. In addition, studies by our research group demonstrate that maternal low protein diet (LPD) impacts development and increases the incidence of prostatic lesions in the offspring of old rats. The molecular mechanisms that trigger these changes are still poorly understood. Thus, in this study was to identify the global expression profile of messenger RNAs (transcriptome) and proteins (proteome) in ventral prostate (VP) samples from young rats submitted to maternal LPD during gestation and lactation, and to integrate molecular pathways involved in prostate development in normal and restricted animals. We used male *Sprague Dawley* rats on the postnatal day (PND) 21 born to dams fed standard diet (17% protein) (control group -CTR), or dams fed LPD (6% protein) during gestation and lactation (gestation and lactation LPD group -GLLP). After this period, the animals were euthanized with anesthetic overdose, weighed and the VP collected. We observed a delay in the stages of prostate development (lower body weight and PV weight) at birth on PND 1), accompanied by disbalance hormonal (increased levels of cholesterol, testosterone and estrogen on PND 21). Proteome analyzes (LC-MS/MS) revealed changes in the pretein profile of the prostate gland in these animals, showing enrichment of molecular pathways associated with the estrogenic disturbance and cancer profile. Transcriptome analysis (HigSeq-2500, Ilumina) revealed a distinct gene profile between the CTR and GLLP groups, showing genes alteraed by maternal LPD were involved in development, cytochrome p450 and cancer pathways. All results were compared to the transcriptome and proteome data for prostate adenocarcionoma (PRAD) in The Cancer Genome Atlas (TCGA) database, with the aim of investigating whether maternal LPD is responsible for altering the gene and protein expression of targets related to the development of lesions prostatic, data associated with the profile of animals in DPN 50 submitted to maternal LPD. Our results highlight that the role of RPM in the normal development of the prostate, altering the molecular pathways that promote the carcinogenesis of the slow-growing prostate with aging. In addition, *in silico* analysis can be a useful tool to characterize potential biomarkers and molecular pathways involved in rodent and human prostate carcinogenesis.

Keywords: Prostate, DoHaD, Transcriptome and Proteome

Lista de Abreviações

AR – Receptor de Andrógeno

CaP- Câncer de Próstata

CTR- Grupo Controle

CYP- Citocromo p450

DNMT- DNA metil transferase

DPN- Dia pós-natal

DHEA- Desidroepiandrosterona

DHT- Di-hidrotestosterona

DOHaD- *Development Origins Heath and Disease*

ERα – Receptor de estrógeno alfa

ERβ – Receptor de estrógeno beta

FOAD – Fetal Origin of Adults Disease

GLLP- Grupo *Gestacional and Lactacional Low Protein*

HPB - Hiperplasia prostática benigna

INCA- Instituto Nacional do Câncer

PIN- neoplasia intraepitelial

PF- Programação Fetal

PV- Próstata ventral

PA- Próstata anterior

PD- Próstata dorsal

PL- Próstata lateral

RPM- Restrição proteica materna

TCGA- *The Cancer Genome Atlas*

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Capítulo 1 - *Revisão de Literatura*

1. Programação Fetal

Nas últimas décadas, tem sido observado aumento exponencial da incidência de doenças relacionadas ao metabolismo corpóreo, principalmente em crianças(AGARWAL et al., 2018; FLODMARK et al., 2004; PENKLER et al., 2019; SEIDELL; HALBERSTADT, 2016). Dentre as mais prevalentes estão a obesidade, diabetes, doenças cardiovasculares, renais e hepáticas (ANTWI, 2008; BIANCO-MIOTTO et al., 2017; DAMASCENO et al., 2014; EMBLETON et al., 2016; GODFREY; BARKER, 2000; LEE, 2015; RODRÍGUEZ-GONZÁLEZ et al., 2015; THORNBURG, 2015). O consumo de alimentos ultraprocessados, hipercalóricos e pouco nutritivos, associado a hábitos de vida sedentário estão intimamente relacionados à pandemia destas doenças (DEARDEN; OZANNE, 2014; FRANK, 2016). Neste contexto, mais evidências epidemiológicas e experimentais demonstram que doenças historicamente consideradas crônicas, podem ter origem a partir de insultos sofridos durante o período embrionário/fetal (AGARWAL et al., 2018; HIVERT et al., 2015; HO et al., 2017; WALKER; HO, 2012). A manutenção de condições adequadas durante o desenvolvimento intrauterino, período de alta vulnerabilidade, é de fundamental importância tanto para a gestante como para o embrião/feto em desenvolvimento (HANSON; GREEN, 2019; MERICQ et al., 2016; SUZUKI, 2018).

Um dos primeiros pesquisadores a demonstrar correlação entre insultos maternos durante a gestação e o aumento de casos de doenças nos descendentes foi o epidemiologista inglês David Barker. Em seu primeiro estudo, Barker e seus colaboradores coletaram dados de recém-nascidos (entre os anos de 1911 e 1930) em um hospital na região de Hertforshire, Inglaterra. O total de mortes por doença cardíaca na idade adulta em indivíduos com baixo peso corpóreo para 1 ano de idade foi 2-7 vezes maior que indivíduos com peso considerado normal (BARKER et al., 1989). Na década de 1980, Barker e colaboradores demonstraram correlação entre a nutrição infantil, incidência de mortalidade neonatal e de mortes por doenças cardíacas na idade adulta em regiões mais pobres da Inglaterra e do País de Gales (BARKER; OSMOND, 1986), criando a "Hipótese de Barker".

Em um outro estudo, Barker coletou dados de gestantes e recém nascidos na região de Lancashire, Inglaterra, nascidos entre os anos de 1935 e 1944. Analisando dados de peso corpóreo ao nascimento, peso da placenta, peso corpóreo e pressão arterial na idade adulta, Barker e colaboradores demonstraram que indivíduos com baixo peso ao nascimento associado a maior tamanho de placentas apresentaram maior pressão arterial (BARKER et al., 1990), dando origem à *"Fetal Origin of Adult Diseases"* (FOAD) (BARKER et al., 1990). Esta teoria, inicialmente controversa, despertou grande interesse médico-científico e anos mais tarde foi criada uma sociedade internacional, denominada Origem desenvolvimentista da Saúde e da Doença ("Developmental Origins Of Health and Disease") (DOHaD) (GLUCKMAN; BUKLIJAS; HANSON, 2015; SUZUKI, 2018) que conta com instituições de pesquisa e de apoio de políticas públicas da Europa, América do Norte e regiões do Pacífico, bem como os países em desenvolvimento (SCHULZ, 2010)(SCHULZ, 2010b).

Em uma das poucas oportunidades de se avaliar a influência de fatores ambientais sobre o desenvolvimento humano, Ravelli et al. (RAVELLI; STEIN; SUSSER, 1976) (1976) demonstraram o

impacto da restrição alimentar sobre os filhos de gestantes expostas a um período de escassez alimentar severa durante o cerco alemão à Holanda, no final da 2a Guerra Mundial (1944-1945), episódio conhecido como *Dutch Hunger Winter* (Inverno da Fome Holandesa). Durante esse período, o consumo alimentar foi limitado a 400-800 calorias/dia, inclusive para gestantes. Esses indivíduos, cujas mães sofreram restrição alimentar nesse período, apresentaram alta incidência de obesidade na vida adulta. Esse evento, embora trágico, proporcionou condições para melhor entendimento dos efeitos da restrição alimentar intrauterina sobre a saúde humana, sendo fundamental para fomentar políticas de atenção à gestante e ao recém-nascido, além de alicerçar o interesse sobre a DOHaD (SCHULZ, 2010).

Apesar dos poucos recursos tecnológicos da época, Hales & Barker (1992) propuseram que o principal mecanismo relacionado a estas respostas adaptativas refere-se ao atraso no desenvolvimento das células beta-pancreáticas produtoras de insulina, resultando em alterações metabólicas e maior incidência de diabetes tipo 2 na idade adulta (HALES; BARKER, 1992). Estudos tem proposto que o aumento da incidência de doenças metabólicas se dá através de mecanismos genéticos e epigenéticos de programação intrauterina (BARKER; THORNBURG, 2013; GLUCKMAN; BUKLIJAS; HANSON, 2015; LANGLEY-EVANS, 2019; LEE, 2015).

Grande parte do conhecimento sobre os efeitos da programação fetal deriva de estudos utilizando-se modelos animais, especialmente roedores. Diversos modelos de programação fetal têm sido desenvolvidos nos últimos anos para investigar os impactos de alterações no ambiente intrauterino sobre a prole. Neste contexto, um dos modelos mais utilizados é o consumo de dieta hipoproteica oferecida às ratas durante a gestação e/ou lactação (COLOMBELLI et al., 2017; DE OLIVEIRA et al., 2016; OZANNE, 1999; PETRY et al., 2001; PINHO et al., 2014; RINALDI et al., 2013; SENE et al., 2013; VEGA et al., 2016). Vários autores demonstraram que o consumo de ração hipoproteica está associado ao baixo peso ao nascimento, redução de crescimento de diferentes órgãos, elevação da pressão sistólica, dislipidemia e resistência à insulina em modelos de roedores (DE BRITO ALVES et al., 2016; DE LIMA et al., 2015; FALCÃO-TEBAS et al., 2012; OZANNE; HALES, 2004; PAULINO-SILVA; COSTA-SILVA, 2016).

Além disso, estudos demonstram que o status nutricional durante períodos críticos de desenvolvimento tem forte impacto sobre a longevidade. Assim, filhotes machos nascidos de ratas alimentadas com dieta hipoproteica durante a gestação e que apresentaram menor peso ao nascimento demonstraram ganho de peso acelerado (catch-up growth) associado ao desenvolvimento de resistência à insulina e diabetes tipo 2 na idade adulta, diminuindo a longevidade (EMBLETON et al., 2016b; MCMILLEN; ADAM; MÜHLHÄUSLER, 2005; PETRY et al., 2001). Estes efeitos podem se agravar quando associados a condições pós-natal desfavoráveis, tais como consumo de alimentos ultraprocessados, hipercalóricos, pouco nutritivos e hábitos de vida sedentários (DEARDEN; OZANNE, 2014). Análises de órgãos específicos revelaram que a RPM durante o período gestacional em ratos acarreta menor número de néfrons no rim (HABIB et al., 2012; SENE et al., 2013), menor quantidade de células beta e de ilhotas de Langerhans no pâncreas (CALZADA et al., 2016; DAHRI et al., 1991), proporção alterada entre os tipos celulares, aumento de estresse oxidativo e fibrose hepática (BURNS et al., 1997; TARRY-ADKINS et al., 2016; VEGA et al., 2016), menor número de neurônios que controlam o apetite no hipotálamo

(PLAGEMANN et al., 2000) e menor número de alvéolos pulmonares (ZANA-TAIEB et al., 2013). Em conjunto, estes dados demonstram que o status nutricional durante o desenvolvimento pré e pós-natal alteram o metabolismo da prole e a morfofisiologia de diferentes órgãos e sistemas, sendo que as consequências podem ser observadas tanto ao nascimento, como a longo prazo.

Zambrano *et al* demonstraram que a restrição proteica durante a gestação foi capaz de reduzir a contagem de espermatozoides e a fertilidade da prole de ratos machos aos DPN 270 (ZAMBRANO et al., 2005). Este mesmo grupo atribuiu estes efeitos à diminuição da quantidade de células de sertoli nos testículos dos animais restritos, o que leva a uma desorganização dos túbulos seminíferos. Além disso, também foram observadas alteração de proliferação e maturação das células germinativas, aumento de estresse oxidativo e perda de defesa antioxidante nos testículos e espermatozóides destes animais (BAUTISTA et al., 2017; RODRÍGUEZ-GONZÁLEZ et al., 2012b, 2014). Em conjunto, estes dados demonstram que a RPM afeta diretamente, não somente a morfogênese, como também a função de órgãos reprodutivos tanto em idade reprodutiva, como também afeta da capacidade reprodutiva em machos durante o envelhecimento (RODRÍGUEZ-GONZÁLEZ et al., 2014).

No sistema genital da prole feminina foram observadas diminuição do número da reserva de folículos ovarianos e alteração no padrão de produção de hormônios esteroides intraovariano (GUZMÁN C, GARCÍA-BECERRA R, AGUILAR-MEDINA MA, MÉNDEZ I, MERCHANT-LARIOS H4 et al., 2014); além do atraso no desenvolvimento mamário (BAUTISTA et al., 2013) e favorecendo o desenvolvimento de câncer de mama após exposição ao desregulador endócrino bisfenol A (BPA) (VARUZZA et al., 2019). Além disso na prole masculina foram descritos diminuição do peso testicular, alteração na organização celular dos túbulos seminíferos, redução da expressão do receptor de andrógeno nos testículos e comprometimento da produção espermática (RODRÍGUEZ-GONZÁLEZ et al., 2012a), seguido de aumento de estresse oxidativo intratesticular e espermático (RODRÍGUEZ-GONZÁLEZ et al., 2014); alteração morfológica e morfométrica do epidídimo e redução da vasculogênese do epidídimo nas fases iniciais do desenvolvimento epididimal e na idade reprodutiva (CAVARIANI et al., 2019; DE MELLO SANTOS et al., 2019).

Atualmente, o conceito *DoHaD* não se trata apenas sobre uma ciência acadêmica, se tornando uma prática no cotidiano dos indivíduos, além da questão sobre saúde pública, formulação de políticas e educação. Estudos relacionados com o tema contribuem para o conhecimento público, a fim de melhorar o nível de saúde humana, principalmente na questão de gerar outra vida, na fase de concepção, gestação e lactação. Com o objetivo de aumentar a conscientização mundial sobre esse conceito, incluindo o público no geral e profissionais da educação e saúde, e da política de saúde, foi lançada uma campanha 'Primeiros 1000 dias', divulgando a importância do estado nutricional de mães que período de amamentação (fetal e neonatal) até 2 anos após o nascimento (total de aproximadamente 1000 dias: 280 dias antes do nascimento + 730 dias infantis após o nascimento). Com isso, as pesquisas do *DoHaD* podem fornecer evidências científicas importantes para a comunidades no geral e científicas, como estímulo para melhor qualidade de vida e bem-estar ao longo das gerações (GLUCKMAN; BUKLIJAS; HANSON, 2015; SUZUKI, 2018).

1.2 Morfofisiologia da próstata: aspectos normais e alterados

A próstata é uma glândula exócrina acessória sexual masculina, cujo desenvolvimento e homeostasia encontram-se sob controle androgênico, responsável pela produção e secreção de fluidos que contribuem para a liquefação do ejaculado, e reprodução (CUNHA et al., 1985). Altamente suscetível à transformação oncogênica a uma frequência significativamente maior que a de outros tecidos sexuais secundários masculinos, como as glândulas seminais (SIEGEL; MILLER; JEMAL, 2018). Ela secreta um complexo proteolítico composto por fosfatase ácida, ácido cítrico, fibrinolisina, enzimas específicas e outros fatores componentes do fluido seminal (MARKER et al., 2003). Esta secreção melhora significativamente a fertilidade masculina, pois liquefaz o ejaculado além de possuir enzimas e gradientes iônicos que alcalinizam o canal vaginal e nutrientes que garantem a motilidade dos espermatozoides (AUMÜLLER; SEITZ, 1990).

Em homens, a próstata é a maior glândula sexual acessória, compartimento subperitoneal, anterior ao reto e inferior a bexiga (VERZE; CAI; LORENZETTI, 2016) . Contém um sistema de ductos ramificados e histologicamente apresenta o epitélio pseudo-estratificado cercado por um estroma fibromuscular anterior que recobre o tecido glandular, o exterior do órgão é envolto por uma capsula fibrosa (HENRY et al., 2018; ITTMANN, 2018; MCNEAL, 1988) Macroscopicamente, a próstata humana é unilobular, contendo três zonas (Figura 1): zona central ramificada anteriormente à uretra prostática, circundando o ducto ejaculatório, equivalendo a 20-25% da próstata; zona de transição, circunda a uretra e compreende cerca de 5-10% do tecido prostático, representa o local da hiperplasia prostática benigna (HPB) (MCNEAL, 1981; TIMMS, 2008); e a zona periférica, que corresponde a 70% do volume da glândula e representa o local mais comum de malignidade (MCNEAL, 1988; TOIVANEN; SHEN, 2017; YACOUB; OTO, 2018).

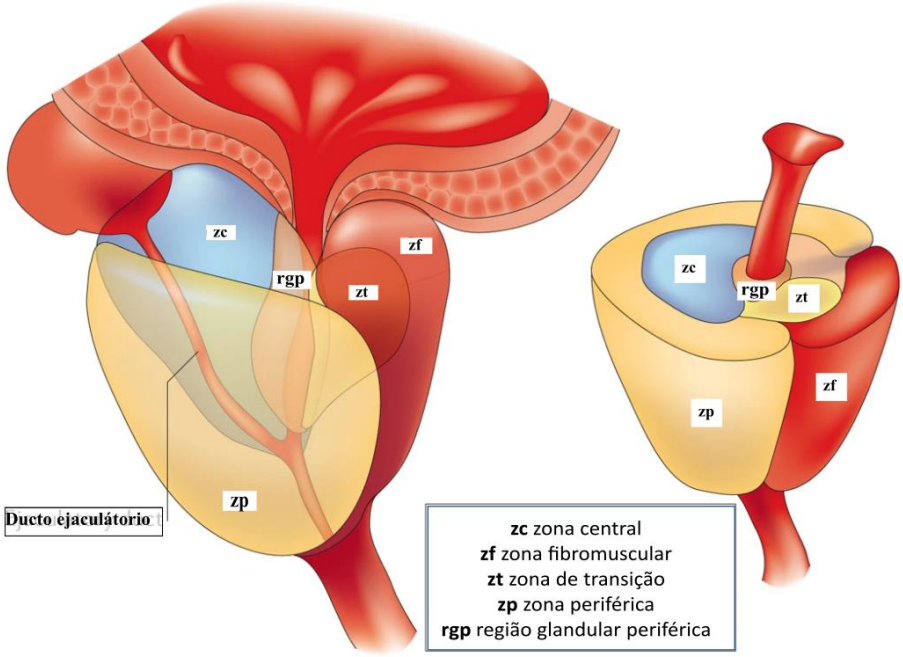

Figura 1. **Anatomia macroscópica e microscópica da próstata humana. A** próstata humana é dividida em três zonas: a zona central, a zona de transição e a zona periférica, representam 20-25%, 5-10% e 70 % do tecido glandular, respectivamente. Uma faixa fibromuscular de tecido separa a zona de transição dos compartimentos glandulares restantes. A zona central se expande em forma de cone ao redor dos dutos ejaculatórios até a base da bexiga. A zona periférica representa a maior parte do tecido glandular prostático e cobre os aspectos posterior e lateral da glândula. Adaptado de Verze, P. (VERZE; CAI; LORENZETTI, 2016).

Em ratos e camundongos a glândula é reconhecida por pares distintos de lóbulos (Figura 2 A) denominados: próstata anterior (PA), próstata ventral (PV), próstata lateral (PL) e próstata dorsal (PD). Se dispõem ao redor da bexiga urinária e se diferenciam pelos padrões de ramificação, histologia e expressão das proteínas secretoras (MARKER et al., 2003; SHIRAI et al., 2000). Os lobos dorsais e lateral podem ser referidos juntos como próstata dorsolateral (PDL).

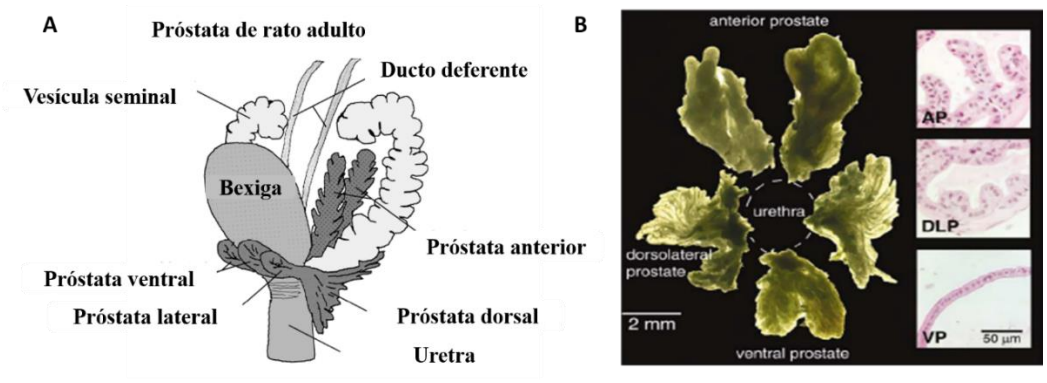

Figura 2. Localização e organização anatômica da próstata de roedores. (A) Vista anterolateral (lado esquerdo) dos lobos da próstata em relação à bexiga, vesículas seminais e uretra, evidenciando os quatro lobos dispostos ao redor da bexiga urinária. (**B)** Aparência macroscópica e organização microscópica (Coloração hematoxilina -eosina H.E.) dos pares de lóbulos prostáticos do rato adulto. (Adaptado de Marker et al., 2003).

Estudos comparativos do desenvolvimento da próstata, demonstraram que a indução da próstata mediada por andrógenos, brotamento epitelial, morfogênese e diferenciação ramificada, são semelhantes na organogênese de roedores e próstata humana(CUNHA et al., 1987, 2018). A morfologia e histologia das próstatas humanas e de roedores também apresentam várias diferenças importantes, além de diferenças na arquitetura dos compartimentos epitelial e estroma. Os tipos de populações celulares são similares e, provavelmente, desempenham as mesmas funções, variando somente na distribuição relativa entre as espécies (IMAMOV et al., 2004; TOIVANEN; SHEN, 2017) (Figura 3). Em humanos, a camada celular basal surge de forma contínua nas seções histológicas, enquanto em roedores, ocorre através de longos processos citoplasmáticos, no qual as células basais entram em contato umas com as outras (HAYWARD; CUNHA; DAHIYA, 1996). Assim, existe uma proporção de 1: 1 de células basais para células luminais na próstata humana, enquanto a proporção em roedores se torna a proporção aproximada de 1: 7 (MCNEAL, 1988; TOIVANEN; MOHAN; SHEN, 2015; TOIVANEN; SHEN, 2017; YACOUB; OTO, 2018). Portanto, este fator favorece estudos referentes à homologia morfofuncional entre as diferentes espécies (IMAMOV et al., 2004; KARR et al., 1995; PRICE, 1963; WANG et al., 2018)

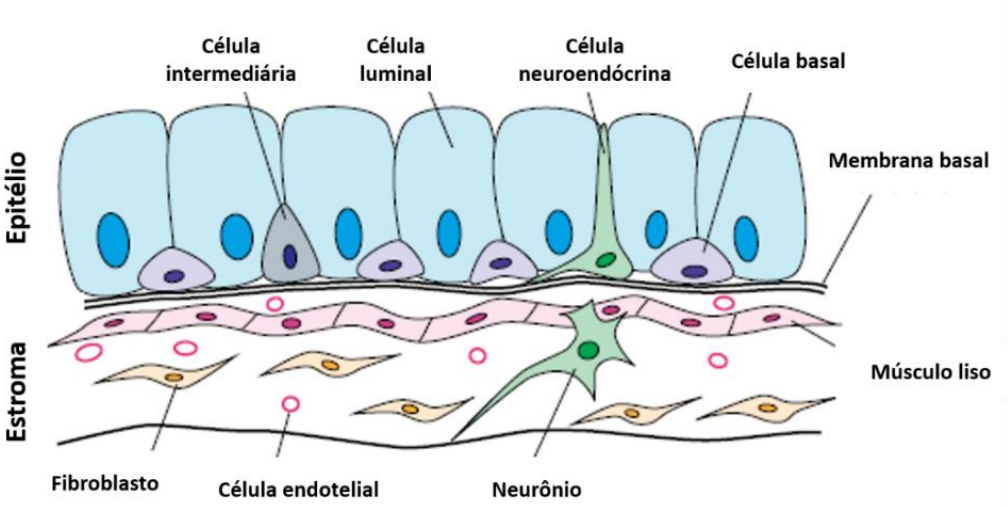

Figura 3. Esquema dos tipos celulares encontrados na próstata adulta. O compartimento epitelial é composto por células basais que revestem a membrana basal, células luminais secretórias e populações raras de células intermediárias e neuroendócrinas. Esses ductos epiteliais são adjacentes a um compartimento estromal que inclui células musculares lisas, fibroblastos e componentes vasculares e neurais. Adaptado de Toivanen (2017).

O desenvolvimento prostático (pré e/ou pós-natal) em roedores tem sido extensivamente utilizado com o objetivo de avaliar os mecanismos moleculares e cascatas de sinalização ativados/inibidos durante a morfogênese glandular (CUNHA et al., 1987; PRINS, 1992; PRINS et al., 2001). O início do desenvolvimento prostático se dá por volta do 17º dia de gestação em camundongos, 18º dia em ratos e entre a 9-10ª semana em humanos (WELSH et al., 2008). Em todas as espécies, inclusive nos humanos, a próstata se desenvolve por meio de um processo altamente conservado, denominado "morfogênese de ramificação", no qual pequenos brotos se projetam a partir do epitélio do seio urogenital (UGS). Enquanto em humanos a maior parte do desenvolvimento da próstata ocorre no período intrauterino, aproximadamente 10 semanas de gestação em humanos (KELLOKUMPU-LEHTINEN; SANTTI; PELLINIEMI, 1980), nos roedores a próstata é rudimentar ao nascimento, sendo a maior parte do desenvolvimento, durante os primeiros 15 dias de vida dia pós-natal (DPN) (TIMMS; MOHS; DIDIO, 1994). Em ambos, esse processo se estende até que a maturidade sexual seja atingida com a puberdade (MARKER et al., 2003; PRINS; PUTZ, 2008) (Figura 4).

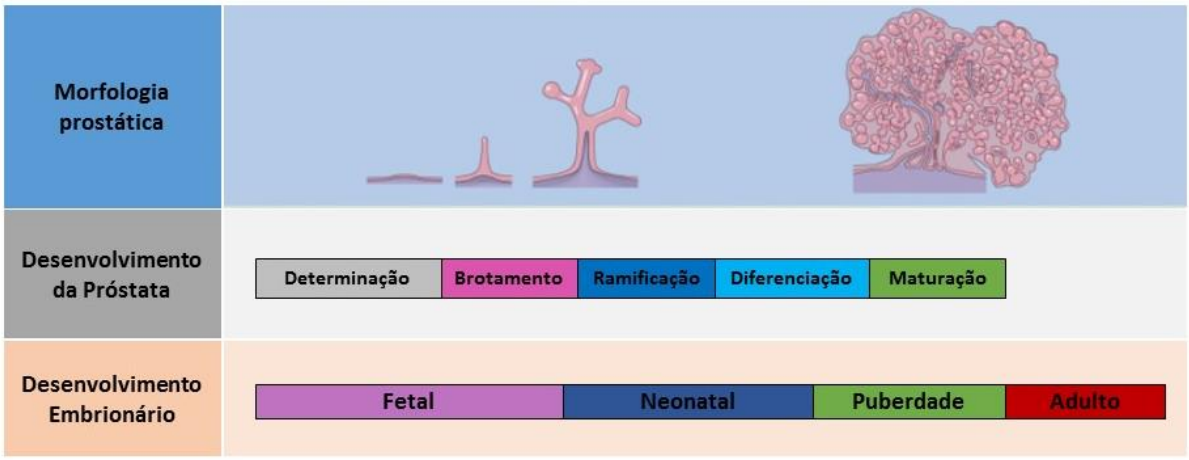

Figura 4. Estágios do desenvolvimento da próstata dos roedores. Adaptado de PRINS; PUTZ, 2008.

Durante o desenvolvimento embrionário, os andrógenos atuam via receptor de Andrógenos (AR), no mesênquima urogenital para induzir a proliferação epitelial, a formação de ductos e a diferenciação das células epiteliais que ocorrem em paralelo ao desenvolvimento do estroma (CUNHA et al., 1992; MARKER et al., 2003; RINALDI et al., 2018). A testosterona, um dos principais andrógenos, é produzida por células intersticiais do túbulo seminífero. Na próstata, a testosterona livre, é convertida em DHT, pela 5 α-redutase do tipo II, mais específica para células epiteliais da próstata. E tanto a testosterona, quanto a DHT podem se ligar ao AR e migrar para o núcleo das células epiteliais (PRINS; BIRCH, 1997; TAPLIN; HO, 2001).

Em resposta aos andrógenos, o epitélio do seio urogenital passa a expressar Sonic hedgehog (Shh), que por sua vez estimula expressão do gene homeobox Nkx3.1 no DG15.5, dois dias antes de surgirem os primeiros brotos prostáticos (WILHELM; KOOPMAN, 2006). Depois do nascimento, ocorre alongamento dos brotos e início da morfogênese com a formação dos ductos (PRINS, 1992). O padrão de ramificação é complexo e lóbulo-específico, sendo que este processo se inicia na próstata ventral (PV) no DPN 3-5 e dois dias depois na próstata dorsolateral (PDL). Nesse período, também ocorre início da diferenciação das células tronco em células basais e luminais, com expressão diferencial de CK e AR, acompanhada do início da formação do lúmen, que atinge as extremidades distais dos ductos no DPN12 (PRINS; BIRCH, 1995) (Figura 5). Concomitantemente, as células mesenquimais prostáticas condensam-se ao redor dos brotos em formação, forma-se uma camada periductal de células musculares lisas enquanto as células interductais diferenciam-se em fibroblastos (HAYWARD; ROSEN; CUNHA, 1997). Outro processo importante que ocorre entre os DPN10-15 é a diferenciação funcional das células epiteliais luminais (PRINS; BIRCH, 1995), sendo que em roedores, as proteínas de secreção prostáticas são detectáveis a partir do DPN20 e tornam-se mais abundantes concomitantemente ao aumento dos níveis séricos de testosterona.

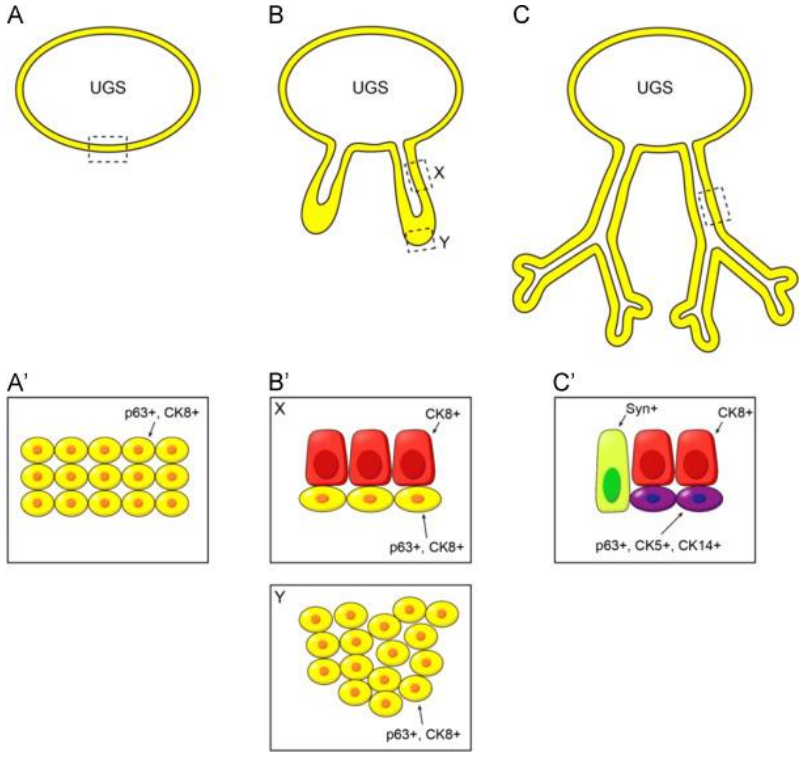

Figura 5. Diagrama ilustrando o desenvolvimento prostático e diferenciação das células epiteliais do camundongo. (A) No dia gestacional 15 (E15), o seio urogenital consiste em epitélio pseudoestratificado e todas as células coexpressam p63 e CK8. (B) No E17, os brotos prostáticos iniciam sua formação. Na região proximal (próximo à uretra, X), as células epiteliais se distinguem em uma linhagem CK8+ revestindo o lúmen e uma linhagem p63+ CK8+ na base do epitélio. Na região distal que ainda não formou luz (Y), as células epiteliais co-expressam p63 e CK8. (C) No DNP1, e se inicia o processo de elongação e ramificação dos ductos. Sinaptofisina (Syn+). Adaptado de PENG; JOYNER, 2015.

Além de ser importante para fertilidade a próstata tem despertado, nos últimos anos, grande interesse médico-científico pela alta incidência de patologias, dentre elas destacam-se a HPB e o CaP (BANERJEE et al., 2018). Segundo dados publicados pela "*Global Cancer Statistics"* (Globocan) 2018, o CaP é o segundo câncer que mais acomete homens no mundo ficando apenas atrás do câncer de pele não melanoma e a quinta principal causa de mortes no mundo, sendo diagnosticados no mundo 1.276.106 novos casos apenas em 2018, mais comum o diagnóstico em países desenvolvidos, atingindo homens com idades acima de 65 anos e mais comum em homens afro-americanos que homens brancos (BRAY et al., 2018). Dados do Instituto Nacional do Câncer (INCA), mostra que a realidade do CaP não é diferente no Brasil, e a estimativa para o ano de 2020 é de 65.840 novos casos (INCA, 2020).

1.3 Próstata e Programação Fetal

Um dos primeiros pesquisadores a considerar as alterações no período intrauterino/perinatal como potencial indutor de desordens prostáticas foi Willian Gardner, professor do Departamento de Patologia da *University of South Alabama*. Em suas observações, Gardner descreveu áreas glandulares com caraterísticas

histopatológicas semelhantes a tecido imaturo na próstata de pacientes, o qual segundo ele, caracteriza um desenvolvimento assincrônico, que pode ter origem a partir da morfogênese prostática e, assim influenciar a biologia da glândula, bem como favorecendo maior incidência de desordens com o envelhecimento (GARDNER, 1995). A partir desta descrição, Gardner propôs a seguinte hipótese: "*The origins of prostatic diseases, including carcinoma, are to be found in the in-utero influences upon the developing prostate*". Como exemplo, Gardner descreveu a exposição de gestantes a fitoestrógenos, que potencialmente poderia influenciar a morfogênese prostática, levando a maior susceptibilidade de desordens com envelhecimento por um mecanismo de imprinting estrogênico (GARDNER, 1995).

A hipótese de Garner alavancou o interesse sobre como o período inicial do desenvolvimento poderia influenciar a incidência de desordens prostáticas. Grande parte do conhecimento existente dos efeitos do ambiente intrauterino/perinatal sobre a biologia prostática foi gerado a partir de estudos experimentais, especialmente em roedores. Dentre os modelos de exposição materna, os comumente utilizados são os seguintes: Diabetes Gestacional (CAMARGO et al., 2017; SANTOS et al., 2014); Desreguladores Endócrinos (SCARANO et al., 2019; VARUZZA et al., 2019); e Restrição Proteica Materna (CAVARIANI et al., 2019; COLOMBELLI et al., 2017; DE MELLO SANTOS et al., 2019; SANTOS et al., 2019).

Especificamente, modelos de dieta hipoproteica revelaram que o desenvolvimento e maturação prostáticos são afetados pela restrição proteica gestacional. Ramos *et al* mostraram menor quantidade de ácinos e tamanho reduzido na próstata dorsolateral de ratos de 21 dias de idade expostos à restrição proteica intrauterina (RAMOS et al., 2010). Rinaldi *et al* obtiveram resultados semelhantes na próstata ventral de ratos submetidos à restrição proteica gestacional e analisados aos 35 dias de vida pós-natal (RINALDI et al., 2013). Além disso, Pinho *et al* também observou atraso no desenvolvimento prostático na prole de animais submetidos ao mesmo protocolo e analisados com 1 dia de vida pós-natal (PINHO et al., 2014).

A restrição proteica materna se tornou um modelo bem estabelecido em nosso laboratório de pesquisa, se dedicando a entender como os efeitos da RPM afetam o desenvolvimento da próstata até o envelhecimento da prole. Rinaldi *et al* (2013) demonstraram que animais submetidos à restrição proteica apenas gestacional, apresentaram prostatite e displasia epitelial nos DPN 120 e 360 com diminuição sérica de di-hidrotestosterona (DHT). Colombelli *et al* observaram que os animais no dia pós-natal 10 (DPN10) apresentaram menor tamanho dos ácinos prostáticos, diminuição da angiogênese da glândula e queda nos níveis séricos de testosterona(COLOMBELLI et al., 2017). Por sua vez, Santos *et al* investigaram o período do desenvolvimento prostático e o envelhecimento, nos animais nos DPN 21 e 540, submetidos à RPM durante a gestação e lactação, destacando o atraso no desenvolvimento da próstata ventral no DPN 21 e maior incidência de lesões prostáticas no envelhecimento, tais como displasia epitelial, neoplasia intraepitelial (PIN) e carcinoma *in situ* (SANTOS et al., 2019). Estes dados foram correlacionados à estrogenização desses animais, devido ao aumento dos níveis séricos de estrógenos nas mães e na prole no DPN 21 e aumento na proporção estrógeno/testosterona nos animais no DPN 540, outro fator importante foi a alteração no perfil protêomico da glândula prostática desses animais, apresentando o

enriquecimento de vias moleculares associadas ao perfil de perturbação estrogênica (SANTOS et al., 2020). Portela *et al* mostraram que esses 2animais no DPN 540 apresentavam um aumento do estresse oxidativo intraprostático e desregulação na expressão de importantes biomarcadores conhecidos do CaP (PORTELA et al., 2021).

Apesar da etiologia complexa do CaP, estudos em modelos animais apontam que exposição a insultos durante o desenvolvimento intrauterino predispõem a glândula a maior incidência de lesões na idade adulta. Dentre estes destacam-se aumento de prostatites e displasias epiteliais seguido de exposição gestacional a dieta hipoproteica (RINALDI et al., 2013), aumento de hipertrofia glandular após exposição gestacional e lactacional à dieta hiperlipídica (PYTLOWANCIV et al., 2016) e desenvolvimento de lesões prostáticas em animais adultos expostos ao bisfenol A ou estradiol no período perinatal (CHEONG et al., 2016b; DE LIMA et al., 2015; HU et al., 2011; RODRÍGUEZ-GONZÁLEZ et al., 2015b).

1.4 Estrógeno e próstata

A biossíntese do estrógeno é catalisada por um membro da superfamília p450, chamada aromatase p450, produto do gene Cyp19 (O'DONNELL et al., 2001). Essa proteína responsável pela ligação ao substrato androgênico e por catalisar uma série de reações, que levam à formação de um anel fenólico no anel A da molécula, característicos dos estrógenos. Em humanos, alguns tecidos como ovário, testículos, placenta, fígado de feto, tecido adiposo, condrócito, osteoblastos (BARKER et al., 2012; O'DONNELL et al., 2001; OLSSON et al., 2007; REDONDO et al., 2000; SAFFARINI et al., 2015) tem capacidade de expressar a aromatase, promovendo a síntese de estrógenos, A próstata também expressa a aromatase, de modo que a síntese de estrógenos está envolvida na indução da HPB (TSUGAYA et al., 1996). Os estrógenos foram caracterizados nos anos 70 (JENSEN et al., 1973), e pertencem à família de receptores nucleares, entre os quais se encontram receptores para hormônios esteroides, vitamina D, retinóides entre outros (O'DONNELL et al., 2001). São conhecidas duas formas de receptores de estrógeno, ER alpha (ERα) e ER beta (ERβ), sendo identificados em camundongos, ratos e humanos (BÖTTNER; THELEN; JARRY, 2014; KUIPER et al., 1996; TREMBLAY et al., 1998).

Os ERs contêm duas formas ativadoras distintas, uma no domínio A/B chamado de *activation function* I (AF I), segunda forma hormônio dependente localizado no domínio E, *activation function* II (AF II), sendo ambas requeridas de forma acoplada para completa ativação dos ERs na maioria das células. Na próstata a localização desses receptores varia, de acordo com o processo fisiológico desencadeado, e o ERα

está localizado nas células estromais enquanto o ERβ nas células epiteliais (PRINS et al., 1998; PRINS; BIRCH, 1997). Os receptores desempenham papeis antagônicos na próstata, sendo o ERα, associado a processos de proliferação celular, enquanto o ERβ desempenha papel antiproliferativo (DA; LU; WANG, 2015; LATIL et al., 2001; STETTNER et al., 2007; ZHAO; ZHOU; GUSTAFSSON, 2019). Prins *et al* (2006) avaliaram a próstata de ratos neonatos expostos a altos níveis de estrógeno, no qual observaram desregulação entre os receptores ERα, ERβ, AR e receptores retinóides (RARs). Além disso, houve alteração da expressão de morfógenos expressos no mesênquima prostático, concluindo-se que altas doses de estrógeno em períodos de desenvolvimento estão relacionadas com alterações estruturais e funcionais na próstata que podem persistir ao longo da vida, causando assim o *imprinting* estrogênico (PRINS et al., 2006).

Em condições normais, o aumento dos níveis de estrógeno intraprostático acontece em dois momentos, no desenvolvimento intrauterino e no envelhecimento (PRINS; KORACH, 2008). O primeiro aumento nos níveis de estrógeno ocorre, em humanos, no terceiro trimestre gestacional, junto a uma queda nos níveis de testosterona, e como consequência há o desenvolvimento de uma metaplasia escamosa no epitélio prostático que regride imediatamente após o nascimento, quando os níveis de estrogênio têm uma queda abrupta (PRINS; KORACH, 2008; WERNERT et al., 1990; ZONDEK et al., 1986). No envelhecimento, o aumento das concentrações séricas de estrógeno está relacionado a maior incidência de lesões prostáticas (KING; NICHOLSON; ASSINDER, 2006; SALINAS et al., 2014; SANTOS et al., 2019).

A exposição a concentrações anormais de testosterona durante a gestação é responsável por alteração na organogênese prostática e alteração no padrão da disposição de marcação positiva para o AR, ERα e em marcadores de proliferação celular na próstata de gerbilos machos e fêmeas ao nascimento (RAMOS et al., 2020). Gomes *et al* (2020) demonstraram que a próstata de gerbilos, ao serem expostos ao alumínio na vida pós-natal, apresentavam um aumento nas concentrações séricas de testosterona e da imunomarcação de células ERα positivas, associando esses resultados a um aumento da proliferação de células epiteliais e estromais, bem como um aumento do compartimento epitelial e diminuição do estroma (GOMES et al., 2020). A partir de dados clínicos de pacientes com CaP, Shen *et al* (2019) estabeleceram que a marcação positiva para integrina α6 (CD49f) e ERα se correlacionavam com o fenótipo de células tronco do câncer de próstata, e que apresentavam um maior potencial metastático de transição epitélio-mesênquima (SHEN et al., 2019). Além disso, o ERα atua em processos inflamatórios e no estabelecimento de lesões pré-neoplásicas (BONKHOFF, 2018).

1.5 Epigenética, Programação Fetal e Estrógeno

A plasticidade e habilidade do embrião/feto em responder as alterações ambientais (dieta, estresse e hormônios), podem ser impactados durante o desenvolvimento intrauterino, sendo capaz de modular a expressão de genes envolvidos com o controle da proliferação e diferenciação celular em uma fase de

morfogênese de órgãos e sistemas vitais (BURTON; FOWDEN; THORNBURG, 2016; SINGH; MORRISON; HOY, 2019; SUZUKI, 2018). Nos últimos anos, mecanismos epigenéticos têm sido apontados como os principais moduladores da expressão gênica envolvidos em várias formas de programação fetal (BIANCO-MIOTTO et al., 2017; GOYAL; LIMESAND; GOYAL, 2019), uma vez que mudanças ambientais podem alterar de forma persistente marcadores epigenéticos e desencadear respostas fenotípicas que afetam a estrutura e funcionamento de órgãos de maneira permanente (SINCLAIR KD, ALLEGRUCCI C, SINGH R, GARDNER DS, SEBASTIAN S, BISPHAM J, THURSTON A, HUNTLEY JF, REES WD, MALONEY CA, LEA RG, CRAIGON J, MCEVOY TG, 2007; WATERLAND; JIRTLE, 2003).

O pesquisador Waddington foi pioneiro em utilizar o termo "epigenética" foi para definir as interações entre genes e o ambiente que podem levar desenvolvimento de um fenótipo diferenciado do indivíduo(WADDINGTON, 1942) . A partir de então, outros autores referem-se à epigenética como mudanças reversíveis e herdáveis no genoma capazes de afetar a expressão gênica e o fenótipo celular, sem, entretanto, alterar a sequência primária de nucleotídeos do DNA (DEANS; MAGGERT, 2015; FERNANDEZ-TWINN; CONSTÂNCIA; OZANNE, 2015). Os principais mecanismos de regulação epigenética incluem metilação de DNA, por enzimas DNA metil transferases (DNMT), modificação pós-transcricional de histonas pelas enzimas histona acetilase (HAC) ou deacelilase (HDAC), além da participação de RNAs não codificantes, dentre estes, os microRNAs (miRNA) que também tem sido descrito como um mecanismo epigenético de regulação da expressão gênica (ALLIS; JENUWEIN, 2016; HEERWAGEN et al., 2010).

Ultimamente, diversos estudos com a temática de estudos de sequenciamento em larga escala baseadas na combinação de “omas” (transcriptoma, MicroRNoma, metiloma e proteoma) tem siso extensivamente publicados (JADDOE, 2019). Além disso, análises integrativas destes dados utilizando ferramentas de bioinformática, associadas a parâmetros clínicos, têm auxiliado na elucidação de redes moleculares envolvidas na patogênese de várias doenças, além de servir como importante ferramenta para identificação e seleção de potenciais alvos terapêuticos (SOOKOIAN et al., 2013). Neste contexto, Esterhuyse *et al* (2015), utilizaram uma análise integrativa do transcriptoma, microRNoma, metiloma e proteoma para demonstrar diferenças em marcadores moleculares em células sanguíneas de pacientes acometidos por tuberculose. Essa estratégia metodológica utilizada pelos autores permitiu a identificação de conjuntos de genes alterados na tuberculose capaz de diferenciarem pacientes saudáveis dos acometidos pela doença (ESTERHUYSE et al., 2015).

Em um modelo de programação fetal induzida por exposição perinatal a desreguladores endócrinos, Cheong et al. (2016), identificaram em próstatas de camundongos recém-nascidos (DPN 3 e 5) regiões promotoras diferencialmente metiladas de aproximadamente 100 genes. A análise de enriquecimento dessas vias demonstrou que 15 desses genes estão envolvidos com a carcinogênese prostática, particularmente com pluripotência de stem cells (Pitx3, Wnt10b, Paqr4, Sox2, Chst14, Tpd52, Creb3l4). Quando estes dados foram comparados com banco de dados da literatura (The Cancer Genome Atlas - TCGA), revelou-se que os mesmos genes estão alterados em pacientes que apresentam recorrência

do CaP. Assim, os autores concluíram que modificações epigenéticas resultantes de exposição perinatal a desreguladores endócrinos podem servir como marcadores preditivos para a recorrência de CaP, além de impactar a carcinogênese prostática com o envelhecimento.

Estudos demonstram que a regulação epigenética pode influenciar em diferentes níveis o crescimento e o desenvolvimento de um organismo (Figura 7) (ALLIS; JENUWEIN, 2016; DELCUVE; RASTEGAR; DAVIE, 2009; KIEFER, 2007). Apesar de intensivos estudos para compreender os mecanismos epigenéticos que geram essas alterações, pouco se sabe sobre os mecanismos moleculares pelos quais essas reações/mudanças químicas são reguladas e/ou como são transmitidas entre as gerações (GOYAL et al., 2014; LONGO; GOYAL, 2014). Estudos em modelo de programação fetal por restrição proteica tem demonstrado a participação de mecanismos epigenéticos, e apesar de existir diversos estudos sobre esse mecanismo no contexto do desenvolvimento embrionário e da biologia do câncer, o conhecimento sobre como a epigenética contribui para a programação fetal ainda são escassos. Por isso a importância para continuar os estudos relacionados com PF e epigenética, uma vez que alterações durante o período preconcepção ou gravidez precoce é a janela de oportunidade para novas estratégias estratégias inovadoras de saúde da população (HEERWAGEN et al., 2010).

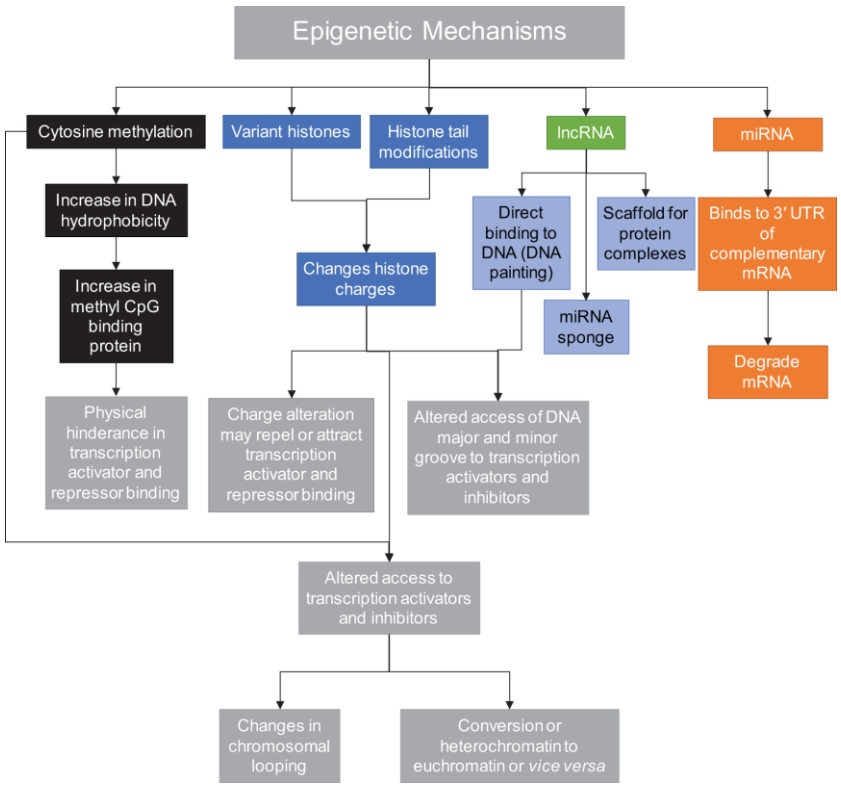

Figura 6. Diferentes mecanismos epigenéticos e processos que regulam a expressão gênica. Adaptado de (D. Goyal et al., 2019).

2. Justificativa e relevância do tema proposto

Nos últimos anos, esforços têm sido destinado ao entendimento de como a exposição a condições gestacionais adversas afetam o desenvolvimento e a saúde dos descendentes. Estudos recentes enfatizam os efeitos danosos da RPM sobre parâmetros metabólicos, doenças cardiovasculares e longevidade da prole. Nosso grupo vem se dedicando a demonstrar o impacto negativo da programação fetal sobre o desenvolvimento e fisiologia prostática, tanto em modelo de diabetes gestacional (CAMARGO et al., 2017; SANTOS et al., 2014), como em modelo de RPM (COLOMBELLI et al., 2017; RINALDI et al., 2013; SANTOS et al., 2019). Atualmente, avanços em tecnologias de sequenciamento associado à maior disponibilidade e barateamento das análises globais, tem permitido uma mudança de paradigma na construção de estudos em biologia molecular e do desenvolvimento. Estas análises integrativas, além de gerarem grande quantidade de dados, permite visão global e integrada dos mecanismos que coordenam a expressão gênica e proteica. Assim, o desenvolvimento deste projeto permitiu uma visão global integrativa dos mecanismos moleculares que controlam tanto o desenvolvimento prostático normal, como em condição de RPM no DPN 21 e 540. Resultados similares do nosso grupo demonstram a maior incidência de lesões prostáticas nos animais restritos (RINALDI et al., 2013; SANTOS et al., 2019), associando com resultados gerados por esse trabalho, juntamente com dados depositados em bancos de dados para modelo de roedor (GeoDataset https://www.ncbi.nlm.nih.gov/gds) ou de humano (TCGA), nos permitirá a caracterização dos possíveis alvos moleculares alterados no início do desenvolvimento que tem relação com a maior susceptibilidade a doenças prostáticas com o envelhecimento nos animais no DPN 540.

3. Objetivo

3.1 Objetivo geral

Caracterizar o perfil global de expressão dos mRNAs e de proteínas na próstata de ratos machos da linhagem *Sprague Dawley* submetidos a restrição proteica materna (gestacional e lactacional), durante a fase de desenvolvimento e envelhecimento.

3.2 Objetivos específicos

- Indução da restrição proteica materna (gestacional e lactacional);

- Caracterizar o perfil global (mRNA e proteína) na próstata ventral (PV) de animais nos 21 dias de idade pós-natal dos - Indução da restrição proteica materna (gestacional e lactacional);

- Correlacionar os dados de expressão global de mRNA (transcriptoma) e de proteínas (proteoma) na PV destes animais;

- A partir destes dados globais, construir redes de interações que apontem quais as vias de sinalização que aparecem alteradas na próstata de ratos submetidos à RPM;

- Correlacionar estes resultados dos alvos diferencialmente expressos a dados de mRNA e proteínas sobre câncer de próstata em humanos (*The Cancer Genome Atlas - Cancer Genome* – TCGA; https://cancergenome.nih.gov/ e *The Human Protein Atlas*; www.proteinatlas.org/) e modelos de roedores (*GeoDataset*- https://www.ncbi.nlm.nih.gov/gds)

4. Resultados

Os resultados obtidos encontram-se descritos nos próximos 2 capítulos (capítulos II e III). O manuscrito referente ao capítulo II *Transcriptomic landscape reveals molecular signaling pathways related to prostate cancer risk in maternal malnourished offspring rats* será submetido ao journal *Frontiers in Cell and Developmental Biology*, com fator de impacto *5.201*. O manuscrito do capítulo III: *Identification of Potential Molecular Pathways Involved in Prostate Carcinogenesis in Offspring Exposed to Maternal Malnutrition*, publicado no periódico Aging, com fator de impacto 5.51.

5. Referências

AGARWAL, P. et al. Maternal obesity, diabetes during pregnancy and epigenetic mechanisms that influence the developmental origins of cardiometabolic disease in the offspring. **Critical Reviews in Clinical Laboratory Sciences**, v. 55, n. 2, p. 71–101, 17 fev. 2018.

ALLIS, C. D.; JENUWEIN, T. The molecular hallmarks of epigenetic control. . 27 jun. 2016, p. 487–500.

ANTWI, S. Malnutrition: missed opportunities for diagnosis. **Ghana medical journal**, v. 42, n. 3, p. 101–104, 2008.

AUMÜLLER, G.; SEITZ, J. Protein secretion and secretory processes in male accessory sex glands. **International review of cytology**, v. 121, p. 127–231, 1990.

BANERJEE, P. P. et al. Androgen action in prostate function and disease. **American journal of clinical and experimental urology**, v. 6, n. 2, p. 62–77, 2018.

BARKER, D. J. et al. Growth in utero, blood pressure in childhood and adult life, and mortality from cardiovascular disease. **BMJ (Clinical research ed.)**, v. 298, n. 6673, p. 564–567, 1989.

BARKER, D. J. et al. Fetal and placental size and risk of hypertension in adult life. **BMJ (Clinical research ed.)**, v.

301, n. 6746, p. 259–62, 4 ago. 1990.

BARKER, D. J.; OSMOND, C. Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. **Lancet (London, England)**, v. 1, n. 8489, p. 1077–81, 10 maio 1986.

BARKER, D. J. P. et al. A possible link between the pubertal growth of girls and prostate cancer in their sons. **American Journal of Human Biology**, 2012.

BARKER, D. J. P.; THORNBURG, K. L. The obstetric origins of health for a lifetime. **Clinical obstetrics and gynecology**, v. 56, n. 3, p. 511–9, 2013.

BAUTISTA, C. J. et al. Protein restriction in the rat negatively impacts long-chain polyunsaturated fatty acid composition and mammary gland development at the end of gestation. **Archives of Medical Research**, v. 44, n. 6, p. 429–436, ago. 2013.

BAUTISTA, C. J. et al. Maternal obesity in the rat impairs male offspring aging of the testicular antioxidant defence system. **Reproduction, fertility, and development**, 9 jan. 2017.

BIANCO-MIOTTO, T. et al. Epigenetics and DOHaD: From basics to birth and beyond. **Journal of Developmental Origins of Health and Disease**, v. 8, n. 5, p. 513–519, 1 out. 2017.

BONKHOFF, H. **Estrogen receptor signaling in prostate cancer: Implications for carcinogenesis and tumor progressionProstate**John Wiley and Sons Inc., , jan. 2018.

BÖTTNER, M.; THELEN, P.; JARRY, H. **Estrogen receptor beta: Tissue distribution and the still largely enigmatic physiological functionJournal of Steroid Biochemistry and Molecular Biology**J Steroid Biochem Mol Biol, , jan. 2014. Disponível em: <https://pubmed.ncbi.nlm.nih.gov/23523517/>. Acesso em: 10 ago. 2020

BRAY, F. et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. **CA: A Cancer Journal for Clinicians**, v. 68, n. 6, p. 394–424, nov. 2018.

BURNS, S. P. et al. Gluconeogenesis, glucose handling, and structural changes in livers of the adult offspring of rats partially deprived of protein during pregnancy and lactation. **The Journal of clinical investigation**, v. 100, n. 7, p. 1768–74, 1 out. 1997.

BURTON, G. J.; FOWDEN, A. L.; THORNBURG, K. L. Placental Origins of Chronic Disease. **Physiological Reviews**, v. 96, n. 4, p. 1509–1565, out. 2016.

CALZADA, L. et al. Maternal protein restriction during gestation impairs female offspring pancreas development in the rat. **Nutrition Research**, v. 36, n. 8, p. 855–862, ago. 2016.

CAMARGO, A. C. L. et al. Streptozotocin-Induced Maternal Hyperglycemia Increases the Expression of Antioxidant Enzymes and Mast Cell Number in Offspring Rat Ventral Prostate. **Anatomical record (Hoboken, N.J. : 2007)**, v. 300, n. 2, p. 291–299, fev. 2017.

CAVARIANI, M. M. et al. Maternal protein restriction differentially alters the expression of AQP1, AQP9 and VEGFr-2 in the epididymis of rat offspring. **International Journal of Molecular Sciences**, v. 20, n. 3, fev. 2019.

CHEONG, A. et al. DNA methylome changes by estradiol benzoate and bisphenol A links early-life environmental exposures to prostate cancer risk. **Epigenetics**, v. 11, n. 9, p. 674–689, 14 set. 2016a.

CHEONG, J. N. et al. Programming of maternal and offspring disease: impact of growth restriction, fetal sex and

503 transmission across generations. . 1 set. 2016 b, p. 4727–4740.

504 COLOMBELLI, K. T. et al. Impairment of microvascular angiogenesis is associated with delay in prostatic
505 development in rat offspring of maternal protein malnutrition. **General and Comparative Endocrinology**, v. 246, p.
506 258–269, 15 maio 2017.

507 CUNHA, G. R. et al. Stromal-epithelial interactions in adult organs. **Cell Differentiation**, v. 17, n. 3, p. 137–148, set.
508 1985.

509 CUNHA, G. R. et al. The endocrinology and developmental biology of the prostate. **Endocrine Reviews**, v. 8, n. 3, p.
510 338–362, 1987.

511 CUNHA, G. R. et al. Normal and abnormal development of the male urogenital tract. Role of androgens, mesenchymal-
512 epithelial interactions, and growth factors. **Journal of andrology**, v. 13, n. 6, p. 465–75, 1992.

513 CUNHA, G. R. et al. **Development of the human prostateDifferentiation**Elsevier Ltd, , 1 set. 2018. Disponível em:
514 <https://pubmed.ncbi.nlm.nih.gov/30224091/>. Acesso em: 12 mar. 2021

515 DA, J.; LU, M.; WANG, Z. Estrogen Receptor Alpha (ERα)-Associated Fibroblasts Promote Cell Growth in Prostate
516 Cancer. **Cell Biochemistry and Biophysics**, v. 73, n. 3, p. 793–798, jun. 2015.

517 DAHRI, S. et al. Islet function in offspring of mothers on low-protein diet during gestation. **Diabetes**, v. 40 Suppl 2,
518 p. 115–20, dez. 1991.

519 DAMASCENO, A. A. et al. Effects of maternal diabetes on male offspring: high cell proliferation and increased activity
520 of MMP-2 in the ventral prostate. **Cell and tissue research**, v. 358, n. 1, p. 257–69, out. 2014.

521 DE BRITO ALVES, J. L. et al. Maternal protein restriction induced-hypertension is associated to oxidative disruption
522 at transcriptional and functional levels in the medulla oblongata. **Clinical and Experimental Pharmacology and**
523 **Physiology**, v. 43, n. 12, p. 1177–1184, dez. 2016.

524 DE LIMA, R. F. et al. Bisphenol-A promotes antiproliferative effects during neonatal prostate development in male
525 and female gerbils. **Reproductive toxicology (Elmsford, N.Y.)**, v. 58, p. 238–45, dez. 2015.

526 DE MELLO SANTOS, T. et al. Maternal Protein Restriction Modulates Angiogenesis and AQP9 Expression Leading
527 to a Delay in Postnatal Epididymal Development in Rat. **Cells**, v. 8, n. 9, p. 1094, set. 2019.

528 DE OLIVEIRA, J. C. et al. Protein Restriction During the Last Third of Pregnancy Malprograms the Neuroendocrine
529 Axes to Induce Metabolic Syndrome in Adult Male Rat Offspring. **Endocrinology**, v. 157, n. 5, p. 1799–812, maio
530 2016.

531 DEANS, C.; MAGGERT, K. A. What do you mean, "Epigenetic"? **Genetics**, 2015.

532 DEARDEN, L.; OZANNE, S. E. The road between early growth and obesity: New twists and turns. **American Journal**
533 **of Clinical Nutrition**, v. 100, n. 1, p. 6–7, 1 jul. 2014.

534 DELCUVE, G. P.; RASTEGAR, M.; DAVIE, J. R. **Epigenetic controlJournal of Cellular Physiology**, 2009.

535 EMBLETON, N. D. et al. Catch-up growth and metabolic outcomes in adolescents born preterm. **Archives of Disease**
536 **in Childhood**, 2016a.

537 EMBLETON, N. D. et al. Catch-up growth and metabolic outcomes in adolescents born preterm. **Archives of Disease**

in Childhood, v. 101, n. 11, p. 1026–1031, 10 nov. 2016b.

ESTERHUYSE, M. M. et al. Epigenetics and Proteomics Join Transcriptomics in the Quest for Tuberculosis Biomarkers. **mBio**, v. 6, n. 5, p. e01187-15, 30 out. 2015.

FALCÃO-TEBAS, F. et al. Maternal low-protein diet-induced delayed reflex ontogeny is attenuated by moderate physical training during gestation in rats. **British Journal of Nutrition**, v. 107, n. 03, p. 372–377, 4 fev. 2012.

FERNANDEZ-TWINN, D. S.; CONSTÂNCIA, M.; OZANNE, S. E. Intergenerational epigenetic inheritance in models of developmental programming of adult disease. **Seminars in Cell and Developmental Biology**, v. 43, p. 85–95, 2015.

FLODMARK, C.-E. et al. New insights into the field of children and adolescents' obesity: the European perspective. **International Journal of Obesity**, v. 28, n. 10, p. 1189–1196, out. 2004.

FRANK, J. Origins of the obesity pandemic can be analysed. **Nature**, v. 532, n. 7598, p. 149, 14 abr. 2016.

GARDNER, W. A. Hypothesis: The prenatal origins of prostate cancer. **Human Pathology**, v. 26, n. 12, p. 1291–1292, dez. 1995.

GLUCKMAN, P. D.; BUKLIJAS, T.; HANSON, M. A. The Developmental Origins of Health and Disease (DOHaD) Concept: Past, Present, and Future. In: **The Epigenome and Developmental Origins of Health and Disease**. [s.l: s.n.].

GODFREY, K. M.; BARKER, D. J. P. **Fetal nutrition and adult disease**. American Journal of Clinical Nutrition. **Anais**...2000

GOMES, L. DA S. et al. Neonatal exposure to aluminum chloride disrupts branching morphogenesis and hormonal signaling of the ventral male prostate and female prostate of gerbils. **Journal of Trace Elements in Medicine and Biology**, v. 61, set. 2020.

GOYAL, D.; LIMESAND, S. W.; GOYAL, R. **Epigenetic responses and the developmental origins of health and diseaseJournal of Endocrinology**, 2019.

GOYAL, R. et al. Cerebral Artery Alpha-1 AR Subtypes: High Altitude Long-Term Acclimatization Responses. **PLoS ONE**, v. 9, n. 11, p. e112784, 13 nov. 2014.

GUZMÁN C, GARCÍA-BECERRA R, AGUILAR-MEDINA MA, MÉNDEZ I, MERCHANT-LARIOS H4, Z. E. et al. Maternal protein restriction during pregnancy and/or lactation negatively affects follicular ovarian development and steroidogenesis in the prepubertal rat offspring. **Archives of Medical Research**, v. 45, n. 4, p. 294–300, maio 2014.

HABIB, S. L. et al. Diabetes and Risk of Renal Cell Carcinoma. **Journal of Cancer**, v. 3, p. 42–48, 2012.

HALES, C. N.; BARKER, D. J. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. **Diabetologia**, v. 35, n. 7, p. 595–601, jul. 1992a.

HALES, C. N.; BARKER, D. J. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. **Diabetologia**, v. 35, n. 7, p. 595–601, jul. 1992b.

HANSON, M.; GREEN, L. A Fetal Origin of Adult Disease. p. 8–19, 2019.

HAYWARD, S. W.; CUNHA, G. R.; DAHIYA, R. Normal development and carcinogenesis of the prostate. A unifying

hypothesis. **Annals of the New York Academy of Sciences**, v. 784, p. 50–62, 30 abr. 1996.

HAYWARD, S. W.; ROSEN, M. A.; CUNHA, G. R. Stromal-epithelial interactions in the normal and neoplastic prostate. **British journal of urology**, v. 79 Suppl 2, p. 18–26, abr. 1997.

HEERWAGEN, M. J. R. R. et al. Maternal obesity and fetal metabolic programming: A fertile epigenetic soil. . set. 2010, p. R711–R722.

HENRY, G. H. et al. A Cellular Anatomy of the Normal Adult Human Prostate and Prostatic Urethra. **Cell Reports**, v. 25, n. 12, p. 3530- 3542.e5, dez. 2018.

HIVERT, M. F. et al. Metabolomics in the developmental origins of obesity and its cardiometabolic consequences. **Journal of developmental origins of health and disease**, v. 6, p. 1–14, 2015.

HO, S. M. et al. Environmental factors, epigenetics, and developmental origin of reproductive disorders. **Reproductive Toxicology**, 2017.

HU, W.-Y. et al. Estrogen-initiated transformation of prostate epithelium derived from normal human prostate stem-progenitor cells. **Endocrinology**, v. 152, n. 6, p. 2150–63, jun. 2011.

IMAMOV, O. et al. Estrogen receptor beta regulates epithelial cellular differentiation in the mouse ventral prostate. **Proceedings of the National Academy of Sciences of the United States of America**, v. 101, n. 25, p. 9375–80, 22 jun. 2004.

INCA. **O Câncer de Próstata| INCA - Instituto Nacional de Câncer**.

ITTMANN, M. Anatomy and histology of the human and murine prostate. **Cold Spring Harbor Perspectives in Medicine**, v. 8, n. 5, p. a030346, maio 2018.

JADDOE, V. W. V. **Translational challenges for the developmental origins of health and disease: Time to fulfill the promises for innovative prevention strategies**. Journal of Developmental Origins of Health and Disease. **Anais**...Cambridge University Press, 1 jun. 2019Disponível em: <https://doi.org/10.1017/S2040174419000242>. Acesso em: 12 mar. 2021

JENSEN, E. V. et al. Transformed estrogen receptor in the regulation of RNA synthesis in uterine nuclei. **Advances in Enzyme Regulation**, v. 11, n. C, p. 1–16, 1973.

KARR, J. F. et al. The presence of prostate-specific antigen-related genes in primates and the expression of recombinant human prostate-specific antigen in a transfected murine cell line. **Cancer research**, v. 55, n. 11, p. 2455–62, 1 jun. 1995.

KELLOKUMPU-LEHTINEN, P.; SANTTI, R.; PELLINIEMI, L. J. Correlation of early cytodifferentiation of the human fetal prostate and leydig cells. **The Anatomical Record**, 1980.

KIEFER, J. C. **Epigenetics in developmentDevelopmental Dynamics**, 2007.

KING, K. J.; NICHOLSON, H. D.; ASSINDER, S. J. Effect of increasing ratio of estrogen:androgen on proliferation of normal human prostate stromal and epithelial cells, and the malignant cell line LNCaP. **Prostate**, v. 66, n. 1, p. 105–114, jan. 2006.

KUIPER, G. G. J. M. et al. Cloning of a novel estrogen receptor expressed in rat prostate and ovary. **Proceedings of the National Academy of Sciences of the United States of America**, v. 93, n. 12, p. 5925–5930, 11 jun. 1996.

LANGLEY-EVANS, S. Nutrigenetics and the early life origins of health and disease. In: **Principles of Nutrigenetics and Nutrigenomics: Fundamentals of Individualized Nutrition**. [s.l: s.n.].

LATIL, A. et al. Evaluation of Androgen, Estrogen (ERα and ERβ), and Progesterone Receptor Expression in Human Prostate Cancer by Real-Time Quantitative Reverse Transcription-Polymerase Chain Reaction Assays. **Cancer Research**, v. 61, n. 5, p. 1919 LP – 1926, mar. 2001.

LEE, H.-S. Impact of Maternal Diet on the Epigenome during In Utero Life and the Developmental Programming of Diseases in Childhood and Adulthood. **Nutrients**, v. 7, n. 12, p. 9492–9507, 17 nov. 2015.

LONGO, L. D.; GOYAL, R. Maternal Stress-Mediated Gene Expression Changes in the Mouse Placenta. In: **The Guide to Investigation of Mouse Pregnancy**. [s.l.] Elsevier, 2014. p. 443–461.

MARKER, P. C. et al. Hormonal, cellular, and molecular control of prostatic development. **Developmental Biology**, v. 253, n. 2, p. 165–174, 2003.

MCMILLEN, I. C.; ADAM, C. L.; MÜHLHÄUSLER, B. S. Early origins of obesity: programming the appetite regulatory system. **The Journal of Physiology**, v. 565, n. 1, p. 9–17, 15 maio 2005.

MCNEAL, J. E. The zonal anatomy of the prostate. **The Prostate**, 1981.

MCNEAL, J. E. Normal histiology of the prostate. **American Journal of Surgical Pathology**, 1988.

MERICQ, V. et al. Long-term metabolic risk among children born premature or small for gestational age. **Nature Reviews Endocrinology**, v. 13, n. 1, p. 50–62, ago. 2016.

O'DONNELL, L. et al. **Estrogen and spermatogenesisEndocrine Reviews**, 2001.

OLSSON, M. et al. Regulation and expression of human CYP7B1 in prostate: Overexpression of CYP7B1 during progression of prostatic adenocarcinoma. **The Prostate**, v. 67, n. 13, p. 1439–1446, 15 set. 2007.

OZANNE, S. E. Programming of hepatic and peripheral tissue insulin sensitivity by maternal protein restriction. **Biochem Soc Trans**, v. 27, n. 2, p. 94–97, fev. 1999.

OZANNE, S. E.; HALES, C. N. Lifespan: Catch-up growth and obesity in male mice. **Nature**, v. 427, n. 6973, p. 411–412, 29 jan. 2004.

PAULINO-SILVA, K. M.; COSTA-SILVA, J. H. Hypertension in rat offspring subjected to perinatal protein malnutrition is not related to the baroreflex dysfunction. **Clinical and experimental pharmacology & physiology**, v. 43, n. 11, p. 1046–1053, nov. 2016.

PENG, Y.-C.; JOYNER, A. L. Hedgehog signaling in prostate epithelial-mesenchymal growth regulation. **Developmental biology**, v. 400, n. 1, p. 94–104, 1 abr. 2015.

PENKLER, M. et al. **DOHaD in science and society: Emergent opportunities and novel responsibilities**. Journal of Developmental Origins of Health and Disease. **Anais**...Cambridge University Press, 1 jun. 2019Disponível em: <https://pubmed.ncbi.nlm.nih.gov/30466503/>. Acesso em: 12 mar. 2021

PETRY, C. J. et al. Diabetes in old male offspring of rat dams fed a reduced protein diet. **International journal of experimental diabetes research**, v. 2, n. 2, p. 139–43, 2001.

PINHO, C. F. et al. Gestational protein restriction delays prostate morphogenesis in male rats. **Reproduction, Fertility**

and Development, v. 26, n. 7, p. 967–973, 2014.

PLAGEMANN, A. et al. Hypothalamic neuropeptide Y levels in weaning offspring of low-protein malnourished mother rats. **Neuropeptides**, v. 34, n. 1, p. 1–6, fev. 2000.

PORTELA, L. M. et al. Increased oxidative stress and cancer biomarkers in the ventral prostate of older rats submitted to maternal malnutrition. **Molecular and Cellular Endocrinology**, v. 523, 1 mar. 2021.

PRICE, D. COMPARATIVE ASPECTS OF DEVELOPMENT AND STRUCTURE IN THE PROSTATE. **National Cancer Institute monograph**, v. 12, p. 1–27, out. 1963.

PRINS, G. S. Neonatal estrogen exposure induces lobe-specific alterations in adult rat prostate androgen receptor expression. **Endocrinology**, v. 130, n. 6, p. 3703–14, jun. 1992.

PRINS, G. S. et al. Estrogen receptor-β messenger ribonucleic acid ontogeny in the prostate of normal and neonatally estrogenized rats. **Endocrinology**, v. 139, n. 3, p. 874–883, 1998.

PRINS, G. S. et al. Influence of neonatal estrogens on rat prostate development. **Reproduction, Fertility and Development**, v. 13, n. 4, p. 241–252, 2001.

PRINS, G. S. et al. The role of estrogens in normal and abnormal development of the prostate gland. **Annals of the New York Academy of Sciences**, v. 1089, p. 1–13, nov. 2006.

PRINS, G. S.; BIRCH, L. The developmental pattern of androgen receptor expression in rat prostate lobes is altered after neonatal exposure to estrogen. **Endocrinology**, v. 136, n. 3, p. 1303–14, mar. 1995.

PRINS, G. S.; BIRCH, L. Neonatal estrogen exposure up-regulates estrogen receptor expression in the developing and adult rat prostate lobes. **Endocrinology**, v. 138, n. 5, p. 1801–1809, 1997.

PRINS, G. S.; KORACH, K. S. **The role of estrogens and estrogen receptors in normal prostate growth and diseaseSteroids**NIH Public Access, , mar. 2008.

PRINS, G. S.; PUTZ, O. **Molecular signaling pathways that regulate prostate gland developmentDifferentiation**, 2008.

PYTLOWANCIV, E. Z. et al. Differential ontogenetic exposure to obesogenic environment induces hyperproliferative status and nuclear receptors imbalance in the rat prostate at adulthood. **The Prostate**, v. 76, n. 7, p. 662–678, maio 2016.

RAMOS, C. DA F. et al. The prostate of weaned pups is altered by maternal malnutrition during lactation in rats. **Asian journal of andrology**, v. 12, n. 2, p. 180–5, 2010.

RAMOS, J. G. et al. Developmental changes induced by exogenous testosterone during early phases of prostate organogenesis. **Experimental and Molecular Pathology**, v. 115, ago. 2020.

RAVELLI, G.-P.; STEIN, Z. A.; SUSSER, M. W. Obesity in Young Men after Famine Exposure in Utero and Early Infancy. **New England Journal of Medicine**, v. 295, n. 7, p. 349–353, ago. 1976.

REDONDO, M. et al. Overexpression of clusterin in human breast carcinoma. **The American journal of pathology**, v. 157, n. 2, p. 393–9, ago. 2000.

RINALDI, J. C. et al. Implications of intrauterine protein malnutrition on prostate growth, maturation and aging. **Life**

Sciences, v. 92, n. 13, p. 763–774, 2013.

RINALDI, J. C. et al. **Maternal protein malnutrition: Effects on prostate development and adult diseaseJournal of Developmental Origins of Health and Disease**Cambridge University Press, , 1 ago. 2018. Disponível em: <https://pubmed.ncbi.nlm.nih.gov/29582717/>. Acesso em: 12 mar. 2021

RODRÍGUEZ-GONZÁLEZ, G. L. et al. Maternal protein restriction in pregnancy and/or lactation affects seminiferous tubule organization in male rat offspring. **Journal of Developmental Origins of Health and Disease**, v. 3, n. 05, p. 321–326, out. 2012a.

RODRÍGUEZ-GONZÁLEZ, G. L. et al. Maternal protein restriction in pregnancy and/or lactation affects seminiferous tubule organization in male rat offspring. **Journal of Developmental Origins of Health and Disease**, v. 3, n. 05, p. 321–326, 8 out. 2012b.

RODRÍGUEZ-GONZÁLEZ, G. L. et al. Accelerated aging of reproductive capacity in male rat offspring of protein-restricted mothers is associated with increased testicular and sperm oxidative stress. **Age (Dordrecht, Netherlands)**, v. 36, n. 6, p. 9721, 2014.

RODRÍGUEZ-GONZÁLEZ, G. L. et al. Maternal obesity and overnutrition increase oxidative stress in male rat offspring reproductive system and decrease fertility. **International Journal of Obesity**, v. 39, n. 4, p. 549–556, abr. 2015a.

RODRÍGUEZ-GONZÁLEZ, G. L. et al. Maternal obesity and overnutrition increase oxidative stress in male rat offspring reproductive system and decrease fertility. **International Journal of Obesity**, v. 39, n. 4, p. 549–556, 15 abr. 2015b.

SAFFARINI, C. M. et al. Developmental exposure to estrogen alters differentiation and epigenetic programming in a human fetal prostate xenograft model. **PloS one**, v. 10, n. 3, p. e0122290, 23 mar. 2015.

SALINAS, C. A. et al. **Prostate cancer in young men: An important clinical entityNature Reviews Urology**Nature Publishing Group, , 2014.

SANTOS, S. A. A. et al. Impact of gestational diabetes and lactational insulin replacement on structure and secretory function of offspring rat ventral prostate. **General and comparative endocrinology**, v. 206, p. 60–71, 15 set. 2014.

SANTOS, S. A. A. et al. Maternal low-protein diet impairs prostate growth in young rat offspring and induces prostate carcinogenesis with aging. **Journals of Gerontology - Series A Biological Sciences and Medical Sciences**, v. 74, n. 6, p. 751–759, 16 maio 2019.

SANTOS, S. A. A. et al. Identification of potential molecular pathways involved in prostate carcinogenesis in offspring exposed to maternal malnutrition. **Aging**, 2020.

SCARANO, W. R. et al. Exposure to an environmentally relevant phthalate mixture during prostate development induces microRNA upregulation and transcriptome modulation in rats. **Toxicological sciences : an official journal of the Society of Toxicology**, jun. 2019.

SCHULZ, L. C. The Dutch Hunger Winter and the developmental origins of health and disease. **Proceedings of the National Academy of Sciences**, v. 107, n. 39, p. 16757–16758, set. 2010a.

SCHULZ, L. C. The Dutch Hunger Winter and the developmental origins of health and disease. **Proceedings of the**

National Academy of Sciences of the United States of America, v. 107, n. 39, p. 16757–8, 28 set. 2010b.

SEIDELL, J. C.; HALBERSTADT, J. The obesity epidemic in the USA — no end in sight? **Nature Reviews Endocrinology**, v. 12, n. 9, p. 499–500, 29 set. 2016.

SENE, A. et al. Impaired cholesterol efflux in senescent macrophages promotes age-related macular degeneration. **Cell metabolism**, v. 17, n. 4, p. 549–61, 2 abr. 2013.

SHEN, Y. et al. Estrogen receptor α-NOTCH1 axis enhances basal stem-like cells and epithelial-mesenchymal transition phenotypes in prostate cancer. **Cell Communication and Signaling**, v. 17, n. 1, maio 2019.

SHIRAI, T. et al. **Experimental prostate carcinogenesis - Rodent modelsMutation Research - Reviews in Mutation Research**, 2000.

SIEGEL, R. L.; MILLER, K. D.; JEMAL, A. Cancer statistics, 2018. **CA: A Cancer Journal for Clinicians**, 2018.

SINCLAIR KD, ALLEGRUCCI C, SINGH R, GARDNER DS, SEBASTIAN S, BISPHAM J, THURSTON A, HUNTLEY JF, REES WD, MALONEY CA, LEA RG, CRAIGON J, MCEVOY TG, Y. L. DNA methylation, insulin resistance, and blood pressure in offspring determined by maternal periconceptional B vitamin and methionine status. **Proceedings of the National Academy of Sciences of the United States of America**, 2007.

SINGH, G.; MORRISON, J.; HOY, W. **DOHaD in Indigenous populations: DOHaD, epigenetics, equity and raceJournal of Developmental Origins of Health and Disease**Cambridge University Press, , 1 fev. 2019. Disponível em: <https://doi.org/10.1017/S2040174419000023>. Acesso em: 12 mar. 2021

SOOKOIAN, S. et al. Fetal metabolic programming and epigenetic modifications: A systems biology approach. **Pediatric Research**, v. 73, n. 4–2, p. 531–542, 2013.

STETTNER, M. et al. The relevance of estrogen receptor-β expression to the antiproliferative effects observed with histone deacetylase inhibitors and phytoestrogens in prostate cancer treatment. **Molecular Cancer Therapeutics**, v. 6, n. 10, p. 2626–2633, out. 2007.

SUZUKI, K. The developing world of DOHaD. **Journal of Developmental Origins of Health and Disease**, v. 9, n. 3, p. 266–269, 2018.

TAPLIN, M. E.; HO, S. M. Clinical review 134: The endocrinology of prostate cancer. **The Journal of clinical endocrinology and metabolism**, v. 86, n. 8, p. 3467–77, ago. 2001.

TARRY-ADKINS, J. L. et al. Coenzyme Q10 prevents hepatic fibrosis, inflammation, and oxidative stress in a male rat model of poor maternal nutrition and accelerated postnatal growth. **The American journal of clinical nutrition**, v. 103, n. 2, p. 579–88, 1 fev. 2016.

THORNBURG, K. L. The programming of cardiovascular disease. **Journal of Developmental Origins of Health and Disease**, v. 6, n. 05, p. 366–376, 2015.

TIMMS, B. G. Prostate development: a historical perspective. **Differentiation**, v. 76, n. 6, p. 565–577, jul. 2008.

TIMMS, B. G.; MOHS, T. J.; DIDIO, L. J. Ductal budding and branching patterns in the developing prostate. **The Journal of urology**, v. 151, n. 5, p. 1427–32, 1994.

TOIVANEN, R.; MOHAN, A.; SHEN, M. M. Basal Progenitors Contribute to Repair of the Prostate Epithelium Following Induced Luminal Anoikis. **Stem Cell Reports**, v. 6, p. 4–11, 2015.

TOIVANEN, R.; SHEN, M. M. Prostate organogenesis: Tissue induction, hormonal regulation and cell type specification. **Development (Cambridge)**, v. 144, n. 8, p. 1382–1398, 2017.

TREMBLAY, G. B. et al. Ligand-independent activation of the estrogen receptors α and β by mutations of a conserved tyrosine can be abolished by antiestrogens. **Cancer Research**, 1998.

TSUGAYA, M. et al. Aromatase mRNA levels in benign prostatic hyperplasia and prostate cancer. **International Journal of Urology**, 1996.

VARUZZA, M. B. et al. Impact of gestational low protein diet and postnatal bisphenol A exposure on chemically induced mammary carcinogenesis in female offspring rats. **Environmental Toxicology**, v. 34, n. 11, p. 1263–1272, nov. 2019.

VEGA, C. C. et al. Resveratrol partially prevents oxidative stress and metabolic dysfunction in pregnant rats fed a low protein diet and their offspring. **The Journal of Physiology**, v. 594, n. 5, p. 1483–1499, 1 mar. 2016.

VERZE, P.; CAI, T.; LORENZETTI, S. **The role of the prostate in male fertility, health and diseaseNature Reviews Urology**Nature Publishing Group, , 1 jul. 2016. Disponível em: <www.nature.com/nrurol>. Acesso em: 12 mar. 2021

WADDINGTON, C. H. The epigenotype. **Endeavour**, 1942.

WALKER, C. L.; HO, S. Developmental reprogramming of cancer susceptibility. **Nature Reviews Cancer**, v. 12, n. 7, p. 479–486, jun. 2012.

WANG, G. et al. **Genetics and biology of prostate cancerGenes and Development**, 2018.

WATERLAND, R. A.; JIRTLE, R. L. Transposable Elements: Targets for Early Nutritional Effects on Epigenetic Gene Regulation. **Molecular and Cellular Biology**, v. 23, n. 15, p. 5293–5300, 1 ago. 2003.

WELSH, M. et al. Identification in rats of a programming window for reproductive tract masculinization, disruption of which leads to hypospadias and cryptorchidism. **Journal of Clinical Investigation**, v. 118, n. 4, p. 1479–1490, 2008.

WERNERT, N. et al. Morphological and immunohistochemical investigations of the utriculus prostaticus from the fetal period up to adulthood. **The Prostate**, v. 17, n. 1, p. 19–30, 1990.

WILHELM, D.; KOOPMAN, P. The makings of maleness: towards an integrated view of male sexual development. **Nature reviews. Genetics**, v. 7, n. 8, p. 620–31, ago. 2006.

YACOUB, J. H.; OTO, A. **MR Imaging of Prostate Zonal AnatomyRadiologic Clinics of North America**W.B. Saunders, , mar. 2018.

ZAMBRANO, E. et al. A maternal low protein diet during pregnancy and lactation in the rat impairs male reproductive development. **The Journal of physiology**, v. 563, n. Pt 1, p. 275–84, 15 fev. 2005.

ZANA-TAIEB, E. et al. Effect of Two Models of Intrauterine Growth Restriction on Alveolarization in Rat Lungs: Morphometric and Gene Expression Analysis. **PLoS ONE**, 2013.

ZHAO, L.; ZHOU, S.; GUSTAFSSON, J.-Å. Nuclear receptors: recent drug discovery for cancer therapies. **Endocrine Reviews**, v. 40, n. 5, mar. 2019.

ZONDEK, T. et al. Hormone levels in the foetal and neonatal prostate. **Acta Endocrinologica**, v. 112, n. 3, p. 447–456, 1986.

Capítulo 2 – Artigo I

Transcriptomic landscape reveals molecular signaling pathways related to prostate cancer risk in maternal malnourished offspring rats

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Abstract

Previous work determined that maternal malnutrition in early life increased the risk of development of prostate cancer in aging in the offspring of rats, as described by Developmental Origins of Health and Disease (DOHaD) concept. Using a model of maternal exposure to low-protein diet (LPD; 6% protein) during the gestational and lactational periods, we characterized the transcriptomic landscape of ventral prostate (VP) in young rats on postnatal day (PND) 21. Here we describe that animals on PND 21 submitted to maternal LPD, had low birth weight, decreased serum levels of progesterone, increased cholesterol, testosterone and estrogen. Through RNA-seq analysis we identified 710 differentially expressed genes (DEGs), significant differences in gene expression profiles were apparent between control vs gestation and lactation maternal LPD group, demonstrated deregulation of key genes, in addition, pathway analysis demonstrated relationship activated in prostate development, as well as during carcinogenesis. Cyp1b1b, Rspo3, Cyp7b1 and Gadd45g were commonly deregulated in young, but also in older maternally malnourished rats, which developed prostate cancer. In silico analysis reveal these four genes can be altered by epigenetic modulation and deregulated in human prostate cancer. Our results described potential key molecular pathways deregulated after exposure to maternal LPD that may connect early life conditions to prostate carcinogenesis in rat offspring. A better understanding of the effects of maternal LPD in development prostate biology may reveal an expansive view of molecular signaling pathways and biomarkers for disorders of the prostate in early life that could persist in adulthood.

Keywords: Prostate, Transcriptome, Developmental process, DOHaD.

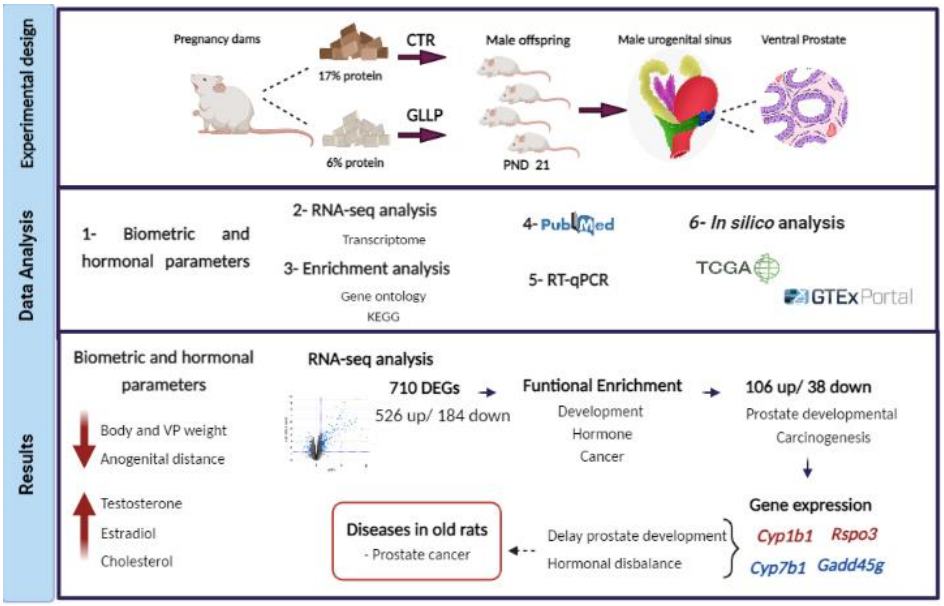

Figure 1. Schematic representation of the experimental design, data analysis, and results. Control group (CTR), Gestation and lactation low protein diet group (GLLP); postnatal- day (PND). Ventral prostate (VP); upregulated genes (up) in red, downregulated genes (down) in blue, differentially expressed genes (DEG).

1. Introduction

Developmental Origins of Health and Disease (DOHaD) states that exposure to inappropriate conditions during periods of high vulnerability, such as intrauterine development, early postnatal life and/or puberty (Suzuki, 2018), can lead to the reorganization and adaptation of cells, making individuals more susceptible to cardiovascular diseases (Godfrey and Barker, 2000), diabetes(Agarwal et al., 2018), and even cancer in adulthood (Welsh et al., 2008). The high vulnerability periods can be affected by several external environmental changes, such as exposure to phthalates(Scarano et al., 2019), maternal obesity(Bautista et al., 2017), and maternal low protein diet (LPD) (Zambrano et al., 2005; Daniel et al., 2016; Santos et al., 2019; Gomes et al., 2020).

Although several studies have described metabolic and cardiovascular diseases as a consequence of early life exposure to adverse conditions (Langley-Evans and Sculley, 2006; Daniel et al., 2016; Vithayathil et al., 2018), the evidence demonstrated that maternal LPD can also negatively impact the male and female genital system (Colombelli et al., 2017), decreasing fertility rate and the onset of puberty (Zambrano et al., 2014). In addition, decreased testicular weight, changes in the cellular organization of seminiferous tubules, reduced androgen receptor expression in the testis and impaired sperm production have also been reported in male offspring exposed to maternal LPD (Rodríguez-González et al., 2012). These results were associated to the increased intratesticular and sperm oxidative stress (Rodríguez-González et al., 2014), morphological and morphometric changes of the epididymis and reduced vasculogenesis of the epididymis in the early stages of epididymal development and accelerated reproductive senescence (Cavariani et al., 2019; de Mello Santos et al., 2019). Experimental evidence has associated maternal LPD during gestation and lactation periods to the increased incidence and severity of prostatic lesions in older offspring rats, results was partially attributed to the estrogenized intrauterine environment, as well as increased estrogen levels in offspring throughout postnatal life (Santos et al., 2019).

The intrauterine/neonatal periods are the key window of vulnerability for prostatic morphogenesis and growth. In rodents, prostate development begins on gestational day (GD) 13 when the androgens synthesized by the fetal testis induce the formation of prostate epithelial buds (Prins and Putz, 2008). It is known that androgens are essential for the development and function of the prostate, thus the prostate gland is hormone-dependent (Prins et al., 1991; Prins and Putz, 2008), such as estrogens play important roles in prostate homeostasis and in diseases (Prins et al., 2006; Nelles et al., 2011). Several decades ago, John McNeal demonstrated prostate gland arises from the endodermal urogenital sinus (UGS) which differs from the rest of the male reproductive accessory sex glands, therewith, proposed that the origins of prostate cancer may have a developmental basis (McNeal, 1981). Prins *et. al* demonstrated that unregulated exposure to estrogen during the development of the prostate, in terms of time, type and dose, can reprogram the gland, lead to differentiation defects and predispose to an increased risk of prostate cancer (Prins et al., 2001; Prins and Ho, 2010). The exposure to high levels of estrogen during critical periods of prostate development has been associated with delay in prostate growth (Ho et al., 2006), changes in the regulation of key molecular signaling pathways

involved in both normal prostate development (Prins and Korach, 2008), as well in the early stages of prostate carcinogenesis (Dobbs et al., 2019). The elucidation of molecular pathways that lead to changes in the prostate development is crucial to understand the role of maternal nutrition on prostate cancer risk in offspring.

Epigenetic mechanisms have long been described as the potential mechanism behind development programming in the context of DOHaD (Barker et al., 1990; Gluckman et al., 2008). Fetal programming demonstrates how events in early life or during the development of the individual, or specific organ, can program that individual for an increased risk of chronic disease later in life (Yan and Yang, 2014; Prins et al., 2018; Felix and Cecil, 2019; Goyal et al., 2019). Animal studies have focused extensively on DNA methylation as the context for epigenetic modification in DOHaD (Bianco-Miotto et al., 2017).Cheong *et. al* demonstrated in rodent models that neonatal exposure to xenoestrogens led to the deregulation of genes related to stem cell function through DNA methylation in the adult prostate, allowing for a prolonged proliferation phase and increasing the number of stem cells stem in the prostate, recognized for malignant transformation induced by carcinogens in adulthood, leading to an increased susceptibility to cancer (Prins et al., 2015, 2017; Cheong et al., 2016).

The present study sought to address critical elements by maternal LPD under prostate biology, across identifying altered genes profile during the development of the offspring rat VP submitted to maternal LPD during gestation and lactation periods. We first undertook an analysis of biometric and hormone parameters of offspring rat submitted to maternal LPD, the analysis included morphology of ventral prostate (VP), body and prostate weight, anogenital distance, total levels of cholesterol, testosterone, and estradiol, parameters this is especially important could serve as markers of maternal LPD. Next, we evaluate changes in the transcriptional profile by RNA-seq analysis, results revealed most deregulated genes related to the developmental process, hormonal, and even cancer. In addition, the most molecular mechanisms associated metabolism of estrogen and androgen, regulation of non-canonical Wnt signaling and p53 signaling pathway, cytochrome P450 metabolism of endogenous sterols and steroid hormone biosynthesis. Finally, translational *in silico* analysis demonstrated that DEGs altered by maternal LPD can be modified via epigenetic modulation, especially four DEGs shared in the VP and human prostate cancer. Overall, these results demonstrated the essential role of maternal malnutrition during early life in modulating gene-environment interaction to increase cancer susceptibility in older rats.

2. Material and Methods

2.1 Experimental groups

Pregnant *Sprague Dawley* rats were divided into two groups (n=12/group): Control (CTR), dams fed a normal protein diet (17% protein); and Gestational and Lactational Low Protein diet (GLLP), dams fed a LPD (6% protein) during gestation and lactation periods. Normal and LPD were provided by PragSoluções (PragSoluções, SP, Brazil). All diets were isocaloric and normosodic (Supplementary

Table 1). The experimental procedures were following the Ethical Principles on Animal Experimentation adopted by the Brazilian College of Animal Experimentation (COBEA) and the protocol was approved by the Ethical Committee on Animal Use of the Institute of Biosciences, São Paulo State University (Protocol: 949/CEUA).

At birthday (PND 1) male offspring were weighed and the anogenital distance (AGD) was measured. The AGD was adjusted to body weight as described by Gallavan *et. al* (Gallavan et al., 1999). The number of pups per litter was reduced to 8 (1:1 ratio between males and females). At PND 21 the AGD was measured, and male pups were euthanized by an overdose of anesthesia (ketamine/xylazine) followed by decapitation, weighing, and the blood, and ventral prostate (VP) were collected and processed by a different analysis as described below.

2.2 Blood serum analysis

Blood samples (n=12/group) were centrifuged (2400 g for 20 minutes) and were used to determine the quantifications of total cholesterol 107 (Labtest®, R76-2/100, Brazil, sensitivity: 0.06 mg/dL), and the circulating concentration of estradiol (17β-estradiol - Monobind®, 4925-300 CA, USA sensitivity: 6.5 pg/mL), testosterone (17β-hdroxy-4-androsten-3-one - Monobind®, 3725-300A, CA, USA. sensitivity: 0.038 ng/mL), using colorimetric methods as manufacturers' protocol.

2.3 Histological procedure

VP samples from CTR and GLLP group (n=8/group) were fixed for 4 hour in Methacarn (70% methanol + 20% chloroform + 10% acetic acid)(Puchtler et al., 1970). After that, the samples were dehydrated in ethanol, diaphanized in xylene, and embedded in Paraplast (Sigma Co, Saint Louis, MO). Sections with 5 µm thickness were produced using a rotative microtome and collected in silanized slides. The slides were stained with hematoxylin-eosin (HE) and used for morphological and stereological analysis. The relative proportions of the ventral prostate (VP) components (epithelium, lumen, and stroma) were determined using stereological analysis describes by Santos et al (Santos et al., 2019). The results were expressed as a percentage of each component and a proportion of the total area analyzed. All analyses were performed using a Leica DMLB 80 microscope connected to a Leica DC300FX camera.

2.4 Total RNA extraction

RNA extraction was performed with Trizol (Ambion, USA) containing 1% of β-Mercaptoethanol, following the manufacturer's instructions. Four VP samples from the CTR group and three VP samples from the GLLP group were used for next-generation sequencing (NGS)-based RNA-seq analysis. The NanoDrop (Thermo Scientific, USA) was used to quantify the total RNA by spectrophotometry, and the

RNA Integrity Number (RIN) was used to measure the RNA quality, using the 2100 Bioanalyzer system (Agilent, USA). Only RNA samples with RIN > 8 were used for subsequent analysis.

2.5 mRNA purification, library construction, and sequencing

HiSeq2500 platform (Illumina) was used for RNAs sequencing. An aliquot of total unfractionated RNA was submitted for library construction, and sequencing Ribo-Zero was used during library preparation for rRNA depletion. Messenger RNA purification and library construction was carried out with total RNA using the TruSeq Standard mRNA Sample Preparation Kit (Illumina), following the manufacturer's specifications. The sequencing was performed with the HiSeq Sequencing System. The entire process of library preparation and sequencing were processed by Macrogen (Seoul, South Korea, Korea) and the data were downloaded through a HTTP link.

2.6 Standardization and identification of DEGs

Initially, the raw data were analyzed in terms of reading quality through FastQC software (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) (Wingett and Andrews, 2018) to inspect for low-quality reads and adapters. Subsequently, the single-end reads were trimmed using the Trimmomatic software (v. 0.36) and default parameters(Bolger et al., 2014). After processing by read-quality media, the remaining reads were aligned against the reference genome of *Rattus Norvegicus* (Genome assembly: Rnor_6.0GCA_000001895.4) obtained through the Ensembl (http://useast.ensembl.org/index.html) through the software STAR (v. 2.5.1a)(Dobin et al., 2013; Dobin, 2016). The resulting files were processed through the Feature Counts software(Liao et al., 2014) to obtain read counts and Cufflinks to obtain fragments per kilobase of transcript per million (FPKM), for specificity analysis of clustering(Trapnell et al., 2010).

Differential expression analysis of RNA-seq data was performed with the edgeR Bioconductor package in R(Chen et al., 2008). Differentially expressed genes (DEGs) between experimental groups were determined applying the statistical cutoff of Log_2 Fold Change $\geq |+0.66| \leq |-0.66|$ and the p-value < 0.05. All different DEGs were showing in Supplementary Table S2.

2.7 Enrichment of ontological terms and molecular pathways

Kobas 3.0 software (http://kobas.cbi.pku.edu.cn/) (Maere et al., 2005) was used to determine the enrichment of ontological terms and molecular pathways related to the identified DEGs on Kyoto Encyclopedia of Genes and Genomes (KEGG) (https://www.genome.jp/kegg/) and Gene ontology (http://geneontology.org/) databases. The cut-off criterion used for both analyses was p-value adjusted < 0.05.

2.8 Identification of shared DEGs between rat VP and samples/patients from PRAD-TCGA

Consistent with the *Early Life Origin of Prostate Cancer* (Gardner, 1995), we investigated whether DEGs-enriched molecular pathways related to the developmental process and carcinogenesis in the VP of young rats could also be deregulated in patients diagnosed with PCa. cBioPortal is a comprehensive web resource that could visualize and analyze multidimensional cancer genomics data (https://www.cbioportal.org/)(Gao et al., 2013), based on the Prostate Adenocarcinoma (PRAD) dataset extracted from The Cancer Genome Atlas (TCGA) database, genetic alterations of DEGs were obtained from cBioPortal. UALCAN (http://ualcan.path.uab.edu/analysis.html), a comprehensive and interactive web resource, provides easy access to publicly available cancer OMICS data (Chandrashekar et al., 2017). In our study, DEGs level was obtained in the "Expression" links using the "TCGA analysis" module and the "PRAD" dataset.

2.9 Identify four protein-coding genes: *Cyp1b1, Rspo3, Cyp7b1, and Gadd45g*

We search across literature the function of DEGs using PubMed (https://pubmed.ncbi.nlm.nih.gov/), the US National Library of Medicine premier bibliographic database. Across the DEGs we selected four protein- coding genes: *Cyp1b1* (Cytochrome P450 Family 1 Subfamily B Member 1), *Rspo3* (R-Spondin 3), *Cyp7b1* (Cytochrome P450 Family 7 Subfamily B Member 1), and *Gadd45g* (Growth Arrest and DNA Damage Inducible Gamma) to validations. In addition, was used the Enrichr tool (https://maayanlab.cloud/Enrichr/) (Kuleshov et al., 2016) to further verify the enrichment of four protein-coding genes (PCG), presented by alluvial diagram, connecting the representative target genes into biological processes and molecular signaling, alluvial plot was generated using SankeyMATIC online tool (http://sankeymatic.com/). Differential expressions of four PCG in prostate tissues were assessed by GEPIA2 (Gene Expression Profiling Interactive Analysis; http://gepia.cancer-pku.cn/)(Tang et al., 2017), an online database with fast and customizable features based on PRAD- TCGA.

2.10 Validation of selected DEGs by RT-qPCR

Aliquots of 2 µg of total RNA from CTR and GLLP groups on PND 21 (n=8/group) were reverse transcribed using the High-Capacity RNA-to-cDNA Kit (Life Technologies), in 10 µl of reaction according to the manufacturer's instructions. The cDNA was then diluted 10X with ultra-pure water and stored at -20°C until used in RT-qPCR reaction. Aliquots of cDNA from each sample were added to a mixture of reagents containing primers "sense" and "anti-sense" and the volume was completed to 10µl with ultrapure water. The primers sequences are shown in Table 1. In addition, we evaluated the expression of these DEGs in VP samples from older offspring submitted to maternal malnutrition. The reactions were performed in duplicates for each target gene in the Real Time QuantStudio 12K flex System (Applied Biosystems) in 384-well plates according to the manufacturer's instructions. The values obtained for all samples were

normalized by the ratio obtained between the informational gene and the reference gene. The values were calculated using the ratio of the GLLP/CTR groups. Relative quantification was performed by the $2^{-\Delta\Delta Ct}$ method(Livak and Schmittgen, 2001)using DataAssistTM v3.01 software (Thermo Fisher Scientific). According to the expression stability among all samples, the reference gene *Gusb* (β-glucuronidase) and *Gapdh* (glyceraldehyde 3-phosphate dehydrogenase) were used to normalize mRNA expression.

Table 1. Specification for Primers used for qRT-PCR.

Gene symbol	Access number	Primer	Amplicon (pb)
CYP7B1	NM_019138.1	AGCTATGGAAGTCCTGCGTG AAGTCTCCTTTCCGCAGACG	2626
GADD45G	NM_001077640.1	GAGTCCGCCAAAGTCCTGAA GTCAATGTCGTTCTCGCAGC	1064
RSPO3	NM_001100990	GGAAAGAATTGGCATGAAGCA CCATAATATCCACTTGGACACGAA	1006
CYP1B1	NM_012940	GGGCTGGATTTGGAGGATGT TCCTGGCTGGCTCCAAAG	2321
GAPDH	NM_017008	GCTCTCTGCTCCTCCCTGTTC GAGGCTGGCACTGCACAA	1306
GUSB	NM_017015	AAATTGTGGCCCGTGGAA GGCACGCGGCACCTTA	2483

Consistent with the early life origins of prostate cancer, we evaluated whether deregulation of selected transcripts observed in the VP of young rats (PND21) persists in older animals (PND 540), contributing to the development of carcinoma in situ, previously described in these animals(Santos et al., 2019, 2020; Portela et al., 2021). For that, we used the RNA samples extracted from the VP of CTR and GLLP groups (n=8/group) of older rats, as published in Santos et al. (2019). The RT-qPCR reactions were carried out as described above.

2.11 Statistical analysis

Bar graphs were performed using GraphPad Prism® software (version 8.00, Graph Pad, Inc., San Diego, CA). The results were submitted to the normalization analysis of the Shapiro-Wilk test. After that, differences of two groups were statistically examined through unpaired tests; the parametric results were submitted to analysis of variance “Test t”, and Mann-Whitney test for nonparametric results. The results were expressed as mean ± SD and differences were considered statistically significant when p-value ≤ 0.05

2.12 In silico analysis of genetic alteration related to selected DEGs in the PRAD-TCGA database

cBioPortal was used to investigate the somatic mutations, copy number variation of shared DEGs identified in our study with those from the tumor samples using the PRAD-TCGA database. Human protein atlas (HPA) project (https://www.proteinatlas.org/humanproteome)(Berglund et al., 2008) was used to demonstrate the tissue localization of these four protein-coding genes in normal and tumor prostate samples by immunohistochemistry.

2.13 Methylation analysis: Global and in silico

Methylated DNA Quantification kit (ab117128, Abcam UK) was used to perform global methylation analysis. For this, we used 80ng aliquots of DNA from each sample (n = 4/group), following the manufacturer's recommendations, were analyzed in 450nm spectrometer. The numerical results obtained regarding the degree of methylation were compared between the CTR and GLLP groups. In addition, cBioPortal was used to correlation between methylation beta values and gene expression profile of shared DEGs identified in our study with those from the tumor samples using the PRAD-TCGA database. UALCAN web resource was used to determine the DNA methylation status of the promoter region of Cyp1b1, RSPO3, Cyp7b1, and Gadd45g genes in normal adjacent tumor tissue and cancer samples extracted from the PRAD-TCGA.

2.13 Data representation and analysis

Heatmaps and Principal Component Analysis (PCA) plots were created using the web tools ClustVis (http://biit.cs.ut.ee/clustvis/) (Metsalu and Vilo, 2015) and Morpheus (https://software.broadinstitute.org/morpheus) (Rn Starruß et al., 2014). Volcano plot generated by ggplot2 on package R.

3. Results

3.1 Maternal malnutrition affects biometric parameters in male offspring

The offspring from the GLLP group showed lower body weight compared to the CTR group on PND 1 (CTR 6.62 ± 0.40 vs GLLP 5.83 ± 0.55) and PND 21 (CTR 39.24 ± 9.37 vs GLLP 20.02 ± 3.87) (Figure 2 A and Table 2). The AGD was reduced in the GLLP group compared to the CTR group (CTR 10.68 ± 0.94 vs GLLP 7.63 ± 1.62) (Table 2). Serum levels of total estrogen (17β-estradiol) (CTR 15.69 ± 1.06 vs GLLP 20.32 ± 3.18), testosterone (17β-hydroxy-4-androstene-3-one) (CTR 0.72 ± 0.11 vs GLLP 1.84 ± 0.29), and cholesterol (CTR 158.7 25.35 ± vs GLLP 792.9 ± 183.9) were increased in GLLP group CTR group, respectively (Table 2).

Table 2. Biometric and hormonal parameters of male offspring on PND21.

Parameters	CTR	GLLP
Dams body weight day 1 pregnancy (g)	232.53±25.28	241.65±27.13
Dams body weight day 21 pregnancy (g)	368.04±16.49	343.76±20.88*
Dams body weight day 1 lactation (g)	238.75±26.50	231.50±24.56*
Dams body weight day 21 lactation (g)	236.76±17.93	193.73±13.00*
Offspring body weight PND 1 (g)	6.62±0.40	5.83±0.55*
Offspring body weight PND 21 (g)	39.24±9.37	20.02±3.87*
Offspring number per dam	10±0.94	10.01±1.16
AGD (mm)	10.68±2.54	7.63±1.62*
Absolute VP weight (mg)	3.20±0.79	1.44±0.38
Relative VP weight	1.15±0.29	0.78±0.15*
Absolute UGC weight (mg)	11.11±1.48	5.6±0.47*
Relative UGC Weight	2.98±0.52	3.12±0.45
IGF-1 (pg/ml)	512.30±65.5	175.30±23.81*
Total cholesterol levels offspring at PND 21 (mg/dL)	158.7 25.35	792.9 ± 183.9*
17β-hydroxy-4-androstene-3-one levels offspring at PND 21 (ng/ml)	0.72±0.11	1.84±0.29*
17β-estradiol levels offspring at PND 21 (pg/ml)	15.69±1.06	20.32±3.18*
Prostate tissue fraction (%)		
Ephitelium	35.71± 7.45	48.05±7.53
Lumen	45.47±12.48	30.50±6.16
Stroma	19.79±6.35	29.64±7.29

Data expressed as mean and standard deviation. * indicates statistical difference between groups when p value ≤0.05.

The absolute and relative VP weight and urogenital complex were decreased in the GLLP group compared to the CTR group (Table 2). In the CTR group, the acini appeared dilated and lined by columnar epithelial cells, with the enlarged lumen and fully with secretion. The fibromuscular stroma was thin around the glandular compartment. In the GLLP group, the luminal acini were reduced, and the stromal compartment was increased compared to the CTR group. These results demonstrated a relationship between hormonal imbalance and impairment of VP growth in the GLLP group (Figure 2 B; Table 2).

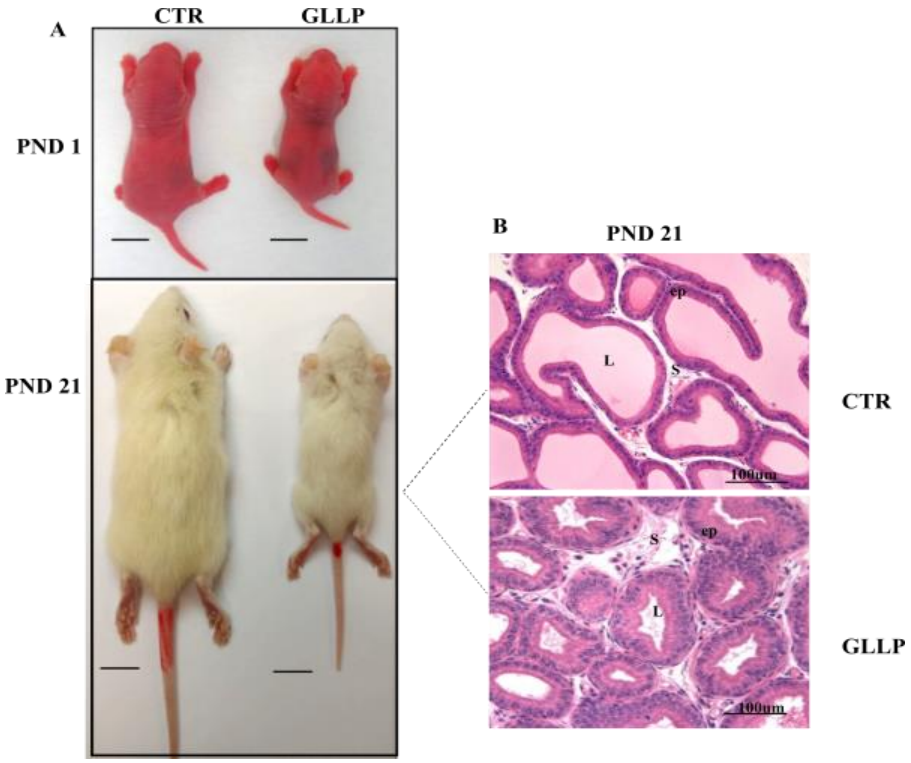

Figure 2. Animals and prostate morphology. **A)** Representative images of male offspring from CTR and GLLP groups on PND 1 and PND 21, scale bars represent 2 cm; **B)** Representative histological sections of ventral prostate (VP) lobes from the CTR and GLLP groups on PND 21 stained with hematoxylin-eosin (HE) for a general view of glandular morphology. L: lumen, ep: epithelium, S: stroma.

3.2 Identification of DEGs between CTR and GLLP groups

The principal component analysis (PCA) showed the sample clusters into the CTR (in red) and GLLP groups (in blue) (Figure 3 A). After data preprocessing and standardization, the edgeR at R package was utilized to screen out DEGs. Based on the threshold of p-value < 0.05 and Log_2 Fold Change ≥ |+0.66| ≤ |-0.66. Figure 3 B shows a volcano plot representing a total of 710 DEGs identified (526 and 184 transcripts, respectively up-or down-regulated). Supplementary table S 2 describes in detail the gene list. Hierarchical clustering of FPKM (fragments per kilobase of transcript per million fragments mapped) values revealed a distinct transcriptomic profile between the animals from CTR and GLLP groups (Figure 3 C). Taken together, these data provide evidence that maternal LPD alters genes profile during the developmental process of the prostate gland, we observed across PCA plot the difference between the samples groups, and clustering by heatmap genes revealed distinct genetic profile between CTR vs GLLP groups.

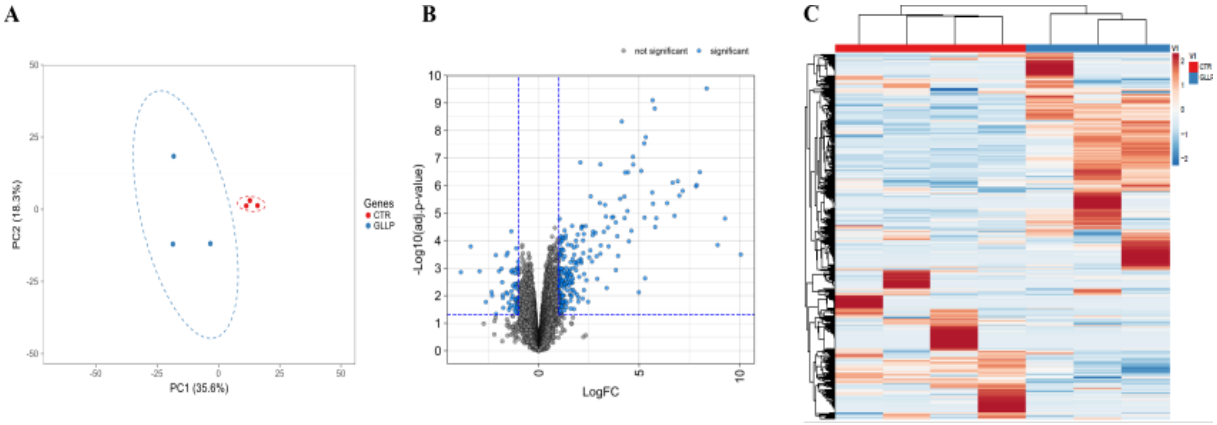

Figure 3. Gene expression analysis in CTR and GLLP groups. A) Principal Component Analysis (PCA) of CTR group in red and GLLP group in blue, visualized distribution between samples; **B)** Volcano plots, each blue point represents the differentially expressed genes between treated group; **C)** Heat map of the mean expression levels (Log_2 Fold Change) of 710 genes differentially expressed between CTR and GLLP groups. The rows (genes expressions) and columns (groups) were clustered using Euclidean distance. The upregulated and downregulated genes with absolute values of Log_2 fold $\leq 0.66 \geq$ and p-value < 0.05. Red color represents high expression and blue, low expression.

3.3 Gene ontology and molecular pathways enriched by DEGs

Upregulated genes enriched ontological terms such as anatomical structure development, multicellular organism development, single-multicellular organism process, single-organism developmental process, developmental process, and molecular pathways involved chemical carcinogenesis, proteoglycans in cancer, Wnt and cadherin signaling, inflammation, and angiogenesis (Figure 4 A, Supplementary Table S 3).The downregulated genes enriched ontological terms related to Ras and Rho GTPase binding, cell differentiation, cellular developmental process, developmental process, anatomical structure development, and molecular pathways associated with p53 signaling and PI3K-Akt signaling pathway (Figure 4 B, Supplementary Table S 3). To correlate early life malnutrition with prostate disorders during aging, we selected 106 upregulated and 38 downregulated genes, which enriched mostly relevant ontological terms and molecular pathways related to prostate developmental biology and cancer for the next analyses. We observed that all DEGs selected from functional enrichment terms (106 upregulated and 38 downregulated genes) presented genetic alterations in 98% (483/498) and 93% (458/498) of queried patients/samples, respectively (Supplementary Figure 1). The most common genetic alterations observed in both datasets were high mRNA expression, amplification and deletion. Besides, all DEGs observed in the VP were also deregulated in human prostate cancer samples, as observed through UALCAN tool (Supplementary Figure S 2).

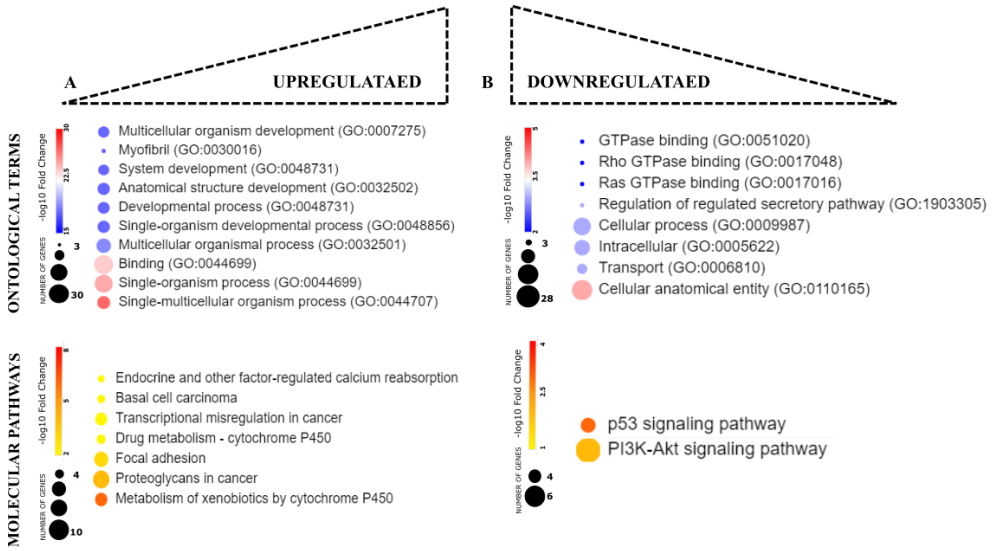

Figure 4. Enrichment pathway analysis of differentially expressed genes in the VP of maternally malnourished offspring rats. Biological processes identified and molecular pathways genes with upregulated genes **(A)** and downregulated genes **(B)**. Significant when $p \leq 0.05$.

3.4 Selection of shared DEGs between VP and prostate cancer samples for validation by RT-qPCR

Based on their enrichment value and key role on prostate developmental biology and carcinogenesis (Supplementary Table S 4) four protein-coding genes: *Cyp1b1* (Cytochrome P450 Family 1 Subfamily B Member 1), *Rspo3* (R-Spondin 3), *Cyp7b1* (Cytochrome P450 Family 7 Subfamily B Member 1), and *Gadd45g* (Growth Arrest and DNA Damage Inducible Gamma) were selected for validation by RT-qPCR in the VP samples. The RT-qPCR confirmed the upregulation of *Cyp1b1*, *Rspo3*, and downregulation of *Cyp7b1* and *Gadd45g* transcripts in the VP of young rats submitted to maternal malnutrition (Figure 5 A-D). Consistent with our hypothesis, *Cyp1b1* and *Rspo3* were upregulated, while *Gadd45g* was downregulated in the VP of older rats submitted to maternal LPD. *Cyp7b1*, which was downregulated on PND 21 showed upregulated in the VP of older rats (Figure 5 E-H). Overall, these results demonstrate the long-term effect of maternal malnutrition on deregulating key molecular signaling pathways involved in both VP development and cancer.

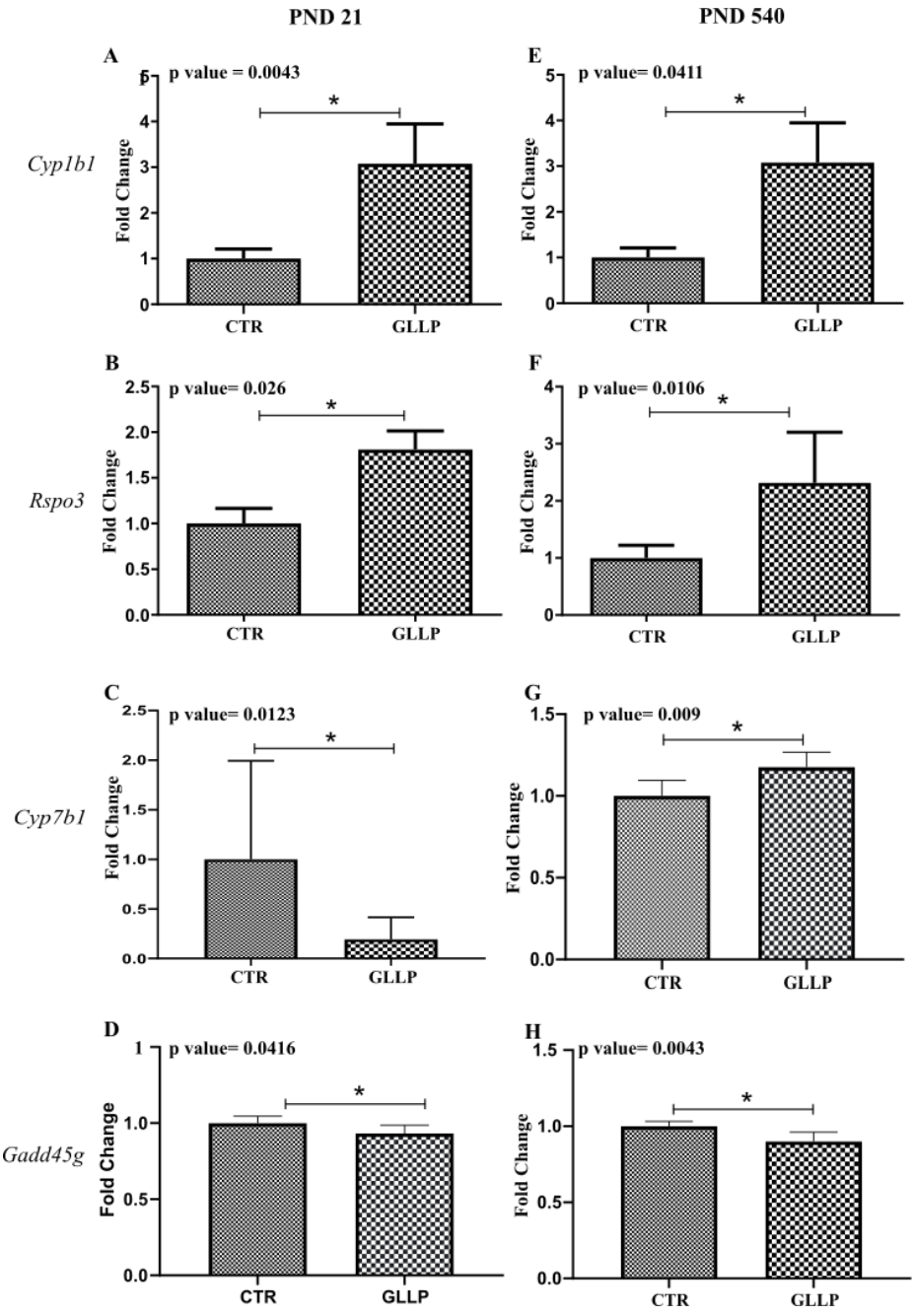

Figure 5. Representative images of RT-qPCR analysis of Cyp1b1 (A and E), Rspo3 (B and F), Cyp7b1 (C and G) and Gadd45g (D and H), in the different experimental groups. CTR = control; GLLP = gestational and lactational protein restriction on postnatal-day 21 and 540, respectively. Indicates statistical difference between groups when $p < 0.05$ (* = $p < 0.05$).

3.5 In silico validation of Cyp1b1, Rspo3, Cyp7b1 and Gadd45g expression in patients with prostate cancer

First, we explored the expression of *CYP1B1, RSPO3, CYP7B1* and *GADD45G* in human prostate cancer and normal samples from PRAD-TCGA and GTEx databases, respectively. As shown in Figure 4 A, *RSPO3* mRNA level was significantly decreased in tumor samples, while *CYP1B1, CYP7B1* and *GADD45G* mRNA level mRNA level was found in tumor tissues, but statistical significance was not reached (Figure 6 A). These genes were able to divide the 497 patients with PCa into two distinct groups, In the survival curve those with high (red line) and low risk (green line), interestingly, these four genes predicted worsen prognosis for patients with prostate cancer, prescribed a significant reduction in life expectancy (hazard ratio = 6.69; log-rank p-value = 0.0022) (Figure 6 B). Although we performed GO and KEGG enrichment terms using KOBAS, the EnrichR database contains additional gene signature collections, consisting in a powerful toll to expanding the terms identified in the enrichment analysis. We used EnrichR for enrichment analysis of *Cyp1b1, Rspo3, Cyp7b1* and *Gadd45g* within the following panel of annotated gene databases (Elsevier Pathway collection, DisGeNET, Disease Pertubation from GEO_up, BioPlanet_2019, KEGG_2019, GO_MF and GO_BP). The main enriched terms were related to male infertility and prostate carcinoma, metabolism of estrogen and androgen, regulation of non-canonical Wnt signaling and p53 signaling pathway, cytochrome P450 metabolism of endogenous sterols and steroid hormone biosynthesis (Figure 6 C, Supplementary Table S 5). These findings showed these four protein-coding genes are distributed among samples adjacent to the tumor and tumors in PCa, beyond are predictors of cancer survival outcomes, in addition, using previously published data reveals association these genes with prostatic disorders.

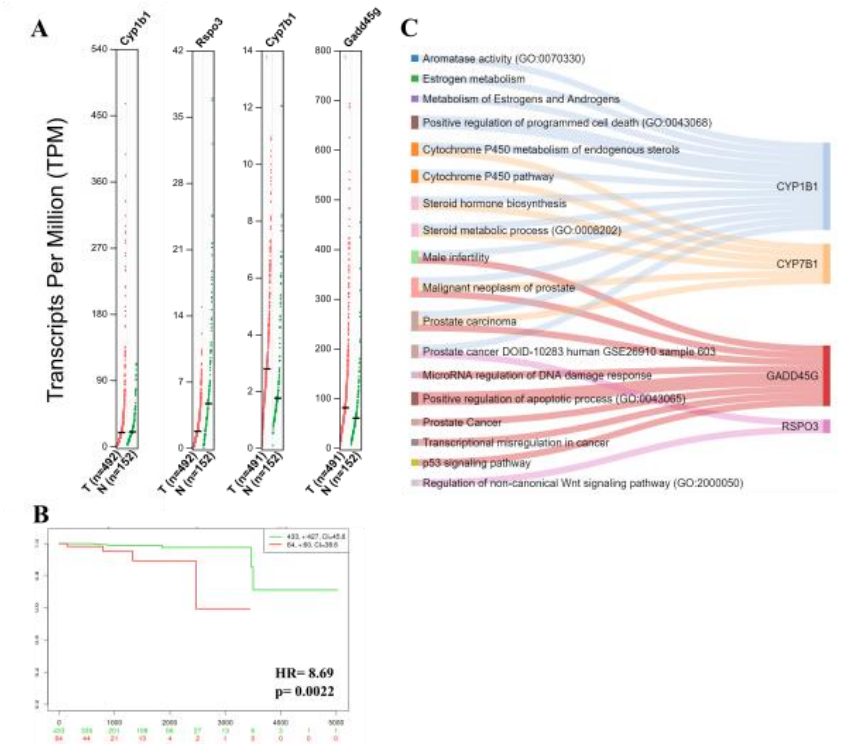

Figure 6. Expression of mRNA levels and Functional enrichment. **A)** Expression mRNA levels of CYP1B1**,** RSPO3, CYP7B1, and GADD45D across prostate cancer samples, tumor (red) and paired adjacent tumor/normal (green); **B)** Overall survival curves comparing patients at high (red line) and low (green line) risks; **C)** Alluvial diagram connecting the representative gene set enrichment analysis tool EnrichR. Alluvial plot was generated using SankeyMATIC online tool (http://sankeymatic.com/).

3.6 Validation across PRAD- TCGA dataset: Genetic alterations and HPA

The oncoprint from CbioPortal was used to demonstrate the main genetic alterations observed in the four targets genes (*CYP1B1*, *RSPO3*, *CYP7B1* and *Gadd45G*) commonly deregulated in the VP and in PRAD-TCGA patients. These genes are altered in 34% (168/491) of queries patients/samples, alterations of *CYP1B1*, *RSPO3*, *CYP7B1* and *Gadd45G*, were found in 8%, 10%, 16%, and 7% of the PCa cases, respectively (Figure 7 A). Altered mRNA expression, amplification, and deep deletion were also commonly observed, suggesting that these set selected genes deregulated our GLLP group, were altered in genetic alterations. Furthermore, we also evaluated the expression profile of the *CYP1B1*, *RSPO3*, *CYP7B1* and *Gadd45G* genes by Human Protein Atlas (HPA) for immunolocalization of the genes in normal and tumor prostate tissue. Correlation analysis based on the levels of mRNA expression of human genes in normal and tumor prostate clinical tissue among DEGs. We observed images with immunostaining low, medium or high with antibodies of the *CYP1B1*, *RSPO3*, and *CYP7B1* genes in both prostate tissues, only *Gadd45G* genes was not detected, and the intensity was negative (Figure 7 B), affirming the presence of these genes in normal and tumor prostate tissue, corroborating our results, the presence of deregulated genes in prostate tissue.

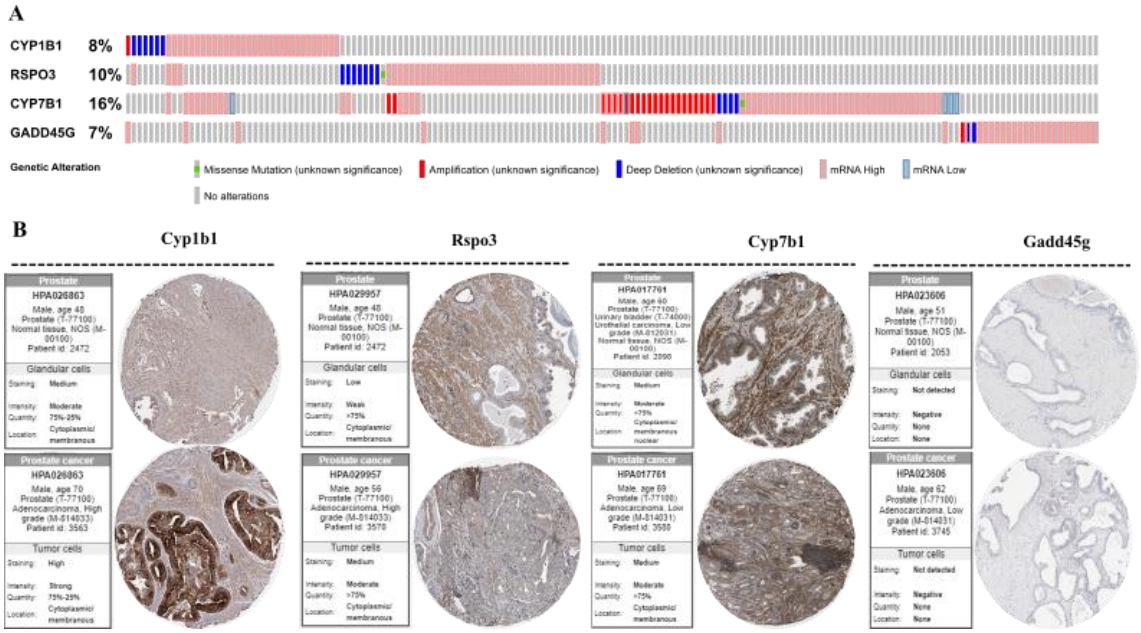

Figure 7. Genetic alterations and gene expression of genes set in TCGA. **A)** Oncoprint data represent Prostate Adenocarcinoma (TCGA, Provisional) samples published in cBioPortal, a visual summary on a query of genetic alteration ofCYP1B1, RSPO3, CYP7B1 and Gadd45G genes in TCGA dataset; **B)** Validation of targets genes by The Human Protein Atlas database.

3.7 Methylation status: mRNA and profile methylation

We performed the global methylation analysis to identify whether the effects of maternal LPD can alter global methylation levels in the offspring's VP submitted to maternal LPD. We observed that methylation levels increased in animals of the GLLP group on PND 21 (Figure 8 A). Additionally, the expression and methylation level of set genes in prostate cancer, identified that all promoter methylation was negatively correlated with the local regulation of all gene expression, with significant values of spearman correlation and p-value of *CYP1B1* (Spearman: -0.77/p = 1.64^{e-95}), *RSPO3* (Spearman: -0.21/p = 4.186^{e-6}), *CYP7B1* (Spearman: -0.36/p = 1.48^{e-16}), and *GADD45G* (Spearman: -0.21/p = 3.510^{e-6}) suggesting the set genes could be methylation silences gene expression (Figure 8 B). Subsequently, we analyzed the expression and promoter methylation level of genes using UALCAN tool from TCGA dataset. We observed the methylation level of Cyp1b1 and Gadd45g (p-value = 25.86^{E-03} and 21.11^{E-07}, respectively) in PCA was significantly lower than normal samples, while the methylation level of *RSPO3* and *CYP7B1* (p-value = $<1^{E-12}$ and 1.62^{E-12}), was higher in cancer than normal samples (Figure 8 C). These data corroborate the changes observed methylation patterns and/or expression of candidate genes, thus, demonstrate that epigenetic mechanisms can act in the modulation of gene expression in animals submitted to maternal LPD in utero environment and during development, may serve as early biomarkers of prostate malignancy.

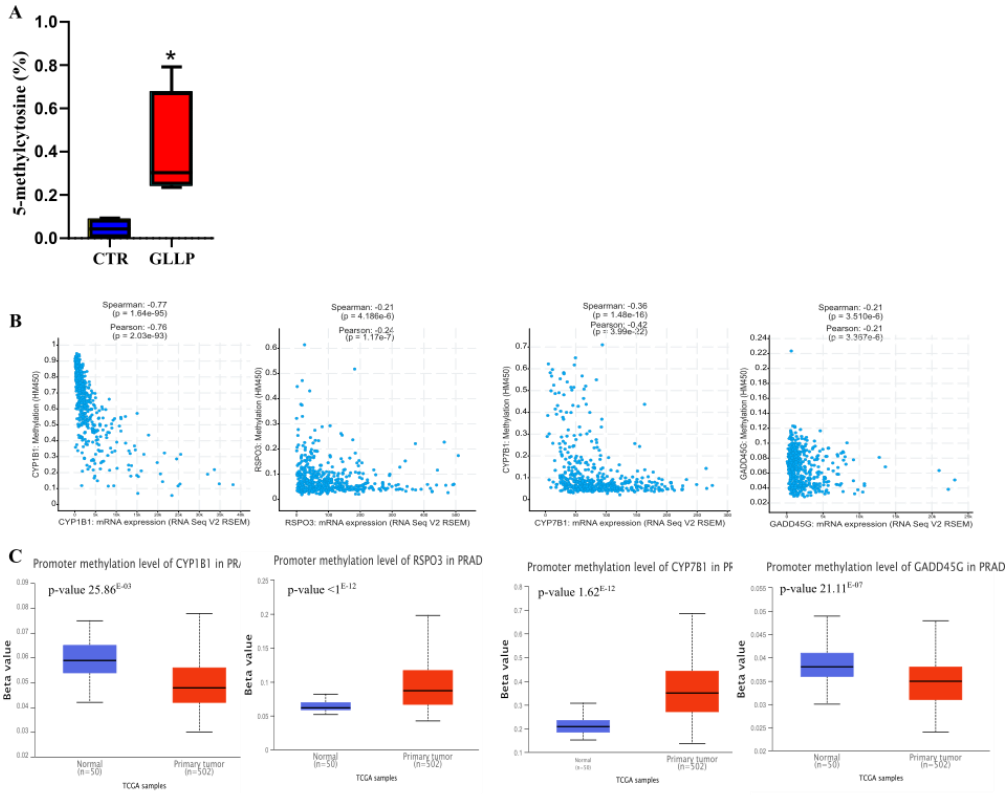

Figure 8. Methylation status in our animals and PRAD-TCGA samples. **A)** Box plot graph showing DNA methylation levels between the CTR and GLLP groups (* significant difference with p ≤0.05). Transcriptional level mRNA expression and promoter methylation of each gene was identified in both cBioPortal **(B)** and UALCAN **(C)** database. p < 0.05 compared with normal prostate tissues.

4. Discussion

In recent years, studies have implicated malnutrition during early life as a key environmental risk factor for the developmental origin of non-communicable chronic diseases, including breast and prostate cancers (Fontelles et al., 2018; Santos et al., 2019; Varuzza et al., 2019; Alcantara Santos et al., 2020). The knowledge of how maternal malnutrition affects key molecular mechanisms involved in prostate developmental biology contributing to carcinogenic in older subjects can help in the identification of non-genomic early origin of PCa, as proposed by Willian Gardner (Gardner, 1995; Bai et al., 2019; Santos et al., 2019). Here we provide evidence that maternal LPD in gestation and lactation periods modulates the expression of genes, our findings identified 526 upregulated and 184 downregulated genes in the VP of maternally malnourished rats on PND 21. In addition, modulates molecular signaling pathways associated metabolism of estrogen and androgen, regulation of non-canonical Wnt signaling and p53 signaling pathway, cytochrome P450 metabolism of endogenous sterols and steroid hormone biosynthesis. Overall, the enrichment analysis reveals deregulation of molecular pathways that participate in both prostate developmental biology, as well as in carcinogenesis in the older subject (Alcantara Santos et al., 2020).

Studies demonstrate that maternal LPD negatively affects the metabolic status, morphogenesis, and homeostasis of important organs and tissues throughout life, which can compromise their capacity and reproductive success [13, 17, 18, 66]. Animals submitted to maternal LPD presented lower birth weight and lower weight gain at weaning, and changes in the gene expression associated with fetus growth (Gonzalez et al., 2016). The AGD is an important biomarker of the response to androgens in the intrauterine period, studies demonstrated that this parameter was regulated by testosterone and DHT produced by fetal testicles (Mitchell et al., 2015). Mitchell *et.al* demonstrated a decrease of testosterone levels in these animals at PND10, which may be associated with the reduction of the AGD, which affects other organs in the male genital system (Mitchell et al., 2015; Diamanti-Kandarakis et al., 2017). A similar response was observed in our findings by Santos *et al*, decrease AGD, and lower body and VP weight (Santos et al., 2019), observed in animals from GLLP group on PND 21. Thus, these events showed that maternal LPD can alters the developmental biology of the progeny, with last long consequences across the life span, such effects can lead to changes in tissue morphology, irreversibly affect the functioning of organs and systems, as well as compromise the longevity of individuals (Qasem et al., 2012; Altobelli et al., 2013).

Research from our laboratory has determined that maternal LPD causes changes in prostate structure, and this a result of direct estrogen actions on prostate gene expression during the critical developmental window (Rinaldi et al., 2013; Borges et al., 2017; Colombelli et al., 2017; Santos et al., 2019; Portela et al., 2021). Similar results were observed by Prins *et. al*, demonstrated a response to high-dose estradiol, androgen receptor levels are suppressed in stromal and epithelial cells due to targeted proteolytic degradation (Prins and Ho, 2010). At the same time, we observed animals from GLLP group on PND 21 an increase in total cholesterol serum levels, followed by high levels of estrogen and testosterone, indicates that, although reduced VP weight, their metabolic status may be altered, and was associated with alteration in the development process of the genital system, such as delay the development of the testis, epididymis, and prostate [25, 26, 57, 67]. These results demonstrated that high-dose estrogen exposures during development, leads to perturbations in steroid-mediated differentiation signals that control prostate development (Prins et al., 2001) , can directly predispose to prostate neoplasia and the formation of tumors with aging (Prins and Ho, 2010; Prins et al., 2017; Santos et al., 2019).

Our animals submitted to maternal LPD on PND 21, presented the upregulated pathways shared in DEGs associated with the developmental process of tissue, cancer, and metabolism cytochrome P450 pathways, whereas downregulated DEGs revealed pathways associated with PI3K-Akt and p53 signaling pathways, Ras-GTPase, and Rho-GTPase binding. Studies demonstrate that P450s are involved biosynthesis of steroid hormones in endogenous signaling and progression of cancer (Omoto et al., 2005; Stiles et al., 2009; Arnold et al., 2010; Hrycay and Bandiera, 2015; Maksymchuk and Kashuba, 2019; Alsubait et al., 2020). The cytochrome P450 monooxygenases (CYPs) acts in the regulation of steroid hormones biosynthesis (Nishimura et al., 2003; McDonnell, PharmD, BCOP and Dang, PharmD, BCPS, 2013; Alex et al., 2016; Ho et al., 2017), and deregulation in this process with increased in estrogen/androgen ratio was associated with the development of human cancers, such as breast and prostate cancers, also, changes in developments gene expression profiles (positive or negative regulation) shown to

be sufficient to mediate tumor promotion (Ao et al., 2007; Franco et al., 2011; Singh et al., 2014). Additionally, inactivating PI3K/AKT signaling inhibits bone metastasis of clinical PCa tissues (Tang et al., 2018), and inactivation of p53 can act to activate *STAT3* and drive prostate tumorigenesis (Pascal et al., 2018), also Ras and Ros-GTPase could modulated p53 signaling (Vallianou et al., 2014). In addition, we observed through oncoprint by cBioPortal that these dysregulated DEGs altered in our animals of the GLLP group, was also involved in the expression profile and genetic alterations were involved in samples of PCa, such as copy number alterations and somatic mutations (Tomlins et al., 2007; Taylor et al., 2010; Berger et al., 2011; Wang et al., 2011; Barbieri et al., 2012; Baca et al., 2013; Cooper et al., 2015; Song et al., 2019). So, the maintenance of these processes are crucial for glandular homeostasis, the terms enrichment analysis showing as possible dysregulation of development pathways, through a hormonal disbalance, main in estrogen, can lead to changes in tissue morphology, irreversibly affect the functioning of organs and systems, as well as compromise the longevity of individuals (Qasem et al., 2012; Altobelli et al., 2013; Dobbs et al., 2019).

To further investigate the role of genes from functional enrichment terms in the developmental process and carcinogenesis, we are applying *in silico* filters to select the four protein-coding genes: *Cyp1b1*, *Rspo3*, *Cyp7b1* and *Gadd45G*. Studies demonstrated that the expression these genes has been found in multiple normal and tumors tissue, including prostate (Olsson et al., 2007; Xu et al., 2015; Mesci et al., 2019; Alsubait et al., 2020). Cytochrome P450 are suggested participated of regulation many process by pathological factors, the expression can be affected by hormones (Daskalopoulos et al., 2012), cytokines (Kot and Daujat-Chavanieu, 2018), pregnancy (Xi et al., 2005), sex and age (Agrawal and Shapiro, 2003; Banerjee et al., 2019). Additionally, all members *RSPO* proteins activate canonical Wnt signaling , and act as antagonize BMP signaling independently (Rong et al., 2014; Lee et al., 2020; Zhang et al., 2020), Wnt signaling are one of the pathways that is of great interest to the PCa community, and BMP signaling the main roles in development, stem cells, adult tissue homeostasis, and disease (Mehta et al., 2011; Lee et al., 2020; Zhang et al., 2020). Other findings, we observed that *Gadd45g* that is involved in diverse processes, including cell cycle, apoptosis, DNA repair, and DNA demethylation(Hollander et al., 1999; Zhan, 2005), also is expressed during mouse embryonic development (Kaufmann et al., 2011). Moreover, expression of these four genes is associated with the recurrence-free survival of PCa patients. These selected protein-coding genes have important functions during the development process and diseases.

Our results demonstrated that the increased levels of testosterone and estradiol in GLLP group on PND 21, could be associated with imbalance between *Cyp1b1* and *Cyp7b1* genes. Studies demonstrated that Cyp P450 was detected on embryonic days 11, 15, and 17 and adult tissues, and was involved in the metabolism of estrogen (Chen et al., 2012; Li et al., 2017; Xu et al., 2019).The CYP1B1 enzyme act in estrogen metabolism by converting into 4-hidroxyestradiol (Gajjar et al., 2012; Gu et al., 2018), whereas CYP7B1 enzyme determine the estrogenic activity by hydroxylation of steroid, 3βAdiol, a metabolite of 5α-dihydrotestosterone (DHT) (Tsuchiya et al., 2005; Lundqvist and Norlin, 2012; Xu et al., 2019; Alsubait et al., 2020). CYP1B1 was detected in many adult and fetal extrahepatic tissues including brain, kidney, prostate, breast, and ocular tissues (Rieder et al., 1998). Besides, other studies showed the CYP1B1 mRNA

have been detected in normal and tumors prostate tissue, however, the levels of CYP1B1 are markedly higher in prostate cancer compared to benign tissues (Carnell et al., 2004; Ragavan et al., 2004; Tokizane et al., 2005; Nishida et al., 2013). However, our GLLP animals on PND 21 presented *Cyp7b1* downregulation expression, these results may be associated with smaller size at birth and lower prostate weight, due to dysregulation on growth pathways. Similar results were observed by Weihua *et. al* suggested that ERβ, 3βAdiol, and *Cyp7b1* are the pathway components that regulates the growth of the rodent VP, this study showed knockouts mice to *CYP7B1*−/− , where the prostate were hypoproliferative before puberty and smaller compared to their wild-type littermates after puberty (Weihua et al., 2002). The opposite happens in the animals from the GLLP group on PND 540, that presented overexpression of *Cyp7b1* gene, Olsson *et. al* hypothesized that *CYP7B1* expression was increased in prostate tumors and that promoter methylation contributes to the regulation of these genes expression in human prostate tissue (Olsson et al., 2007). Santos et. al showed the high estrogen levels observed in the offspring rats on PND 21 and 540, as well as an increase in the estrogen/testosterone ratio, together with the alteration of the Cyp1b1 and Cyp7b1 gene expression, showed in this study, may have been altering the tissue response to hormones in animals submitted to maternal LPD, demonstrates delay in the developmental capacity of the prostate gland, in addition to contributing to carcinogenesis in old rats on PND 540 (Santos et al., 2019, 2020).

Studies demonstrated that RSPO was detected during mouse development [125], besides to positively regulating the WNT/b-catenin signaling, thus, can act on prostate development (Murillo-Garzón and Kypta, 2017; Yeh et al., 2019). Metha *et. al* demonstrated Rspo3 as potential activators of b-catenin signaling in the male UGS, other studies showed that stromal AR regulation may be conserved during prostate development throughout by *Rspo* genes and Wnt pathway (Han et al., 2011; Mehta et al., 2011; Lee et al., 2020). The overexpression of Wnt signaling, by the Wnt agonists Rspo3, has been observed in prostate tumors or stroma. (Zhang et al., 2020). Johnen *et. al* showed that *Gadd45g* was essential for primary sex determination, male fertility and testis development. In this studies with mice with phenotype *Gadd45g*−/− decreased *SRY* expression and blocked *SOX9* expression, also dysregulation in developmental defects other than sex reversal or infertility, and human abnormal appearance of genital organs [30], in addition the dysregulated expression to aberrant promoter methylation has been observed in multiple types of tumors (Ying et al., 2005; Guo et al., 2013; Xu et al., 2015), even PCa (Ramachandran et al., 2009). Thus, these alterations in the Wnt / b-catenin pathway by overexpression of *Rspo3* gene, associated with decreases *Sry* expression by downregulation of *Gadd45g* gene, may explain the deregulated molecular mechanism during prostate development, resulting in a developmental delay in our animals of GLLP group on PND 21, whereas in the GLLP animals on PND 540 the Rspo3 deregulated may be associated stromal cells in the tumor microenvironment and the activating mutations seem to sensitize the tumors responsive Wnt-Rspo (Santos et al., 2019, 2020).

Another interesting observation were global methylation analysis showed increased in GLLP animals on PND 21, these data corroborate the changes observed in mRNA, demonstrating that epigenetic mechanisms can act in the modulation of gene expression in these individuals during development, these

changes in global DNA methylation in our animals in the GLLP group, can be an epigenetic signature associated with increased susceptibility and changes in the gland of young animals, which may persist into adulthood due to maternal LPD exposure. Studies show that neonatal development is a susceptible period for the effects of DNA methylation, considering that a prostate is responsive to estrogen, becoming highly vulnerable to insults by estrogenic agents during development (Prins et al., 2006, 2011; Prins and Ho, 2010). In addition, we observed mRNA and methylation profile in normal and tumor prostate patients/samples PRAD-TCGA, suggested these genes has multiple modes of action, as dysregulation hormone and development pathways, and as well as epigenetic DNA demethylation (Ushijima and Asada, 2010; Guo et al., 2013; Fernandez-Twinn et al., 2015; Cheong et al., 2016). These results clearly show that there is a great deal more to be learned regarding the role of DNA methylation, thus, future studies are planned to develop a panel of methylated genes that may be used as markers across rats submitted to maternal LPD.

In summary, we have shown that the estrogenic environmental caused by maternal LPD during the developmental critic period, results change in important hormone-responsive genes in prostate cells, modifying prostate developmental biology. Furthermore, the present findings provide evidence that animals submitted to maternal LPD have been changes in the expression pattern of genes responsible for several signaling pathways that act during this phase of development, such as decreased body weight and delayed development of prostate biology, in addition to changes in cell proliferation and/or differentiation, which may connect early life exposure to prostate carcinogenesis in rat offspring, besides impacts the prostate epigenome during early life.

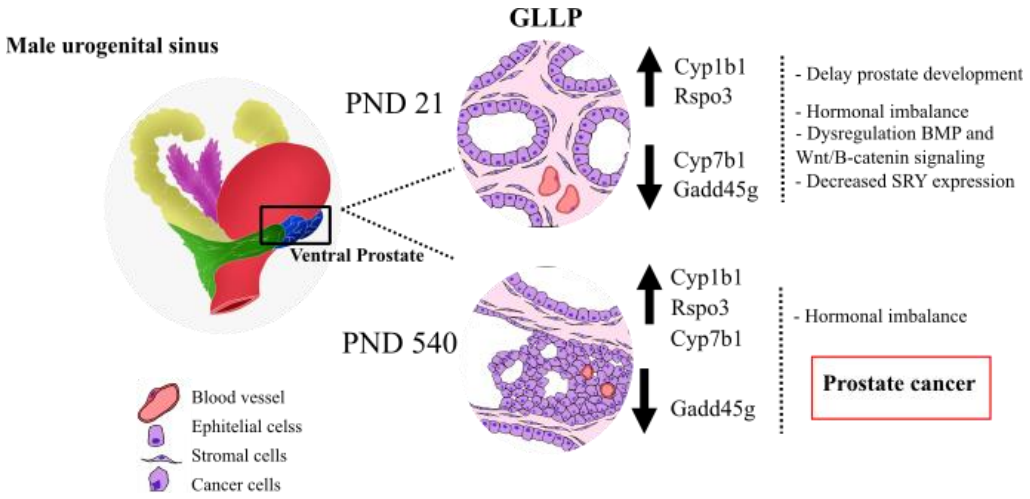

Figure 9. Molecular mechanisms altered by maternal LPD in GLLP animals on PND 21 and 540.

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Conflicts of interest

The authors declare no competing financial interests.

Authors' contributions

Conceptualization, A.C.L.C., and L.A.J.; methodology, A.C.L.C., S.A.A.S., F.B.C., KT.C., L.M.F.P., M.N.F, L.A.B., E.R.D., C.S.M., L.A.J.; formal analysis, A.C.L.C., C.S.M, L.A.J.; investigation, A.C.C., S.A.A.S., L.A.J.; resources, A.C.L.C, S.A.A.S., L.A.J; data curation, A.C.L.C, L.A.J.; writing—original draft preparation A.C.L.C., S.A.A.S., L.A.J.; writing—review and editing, all authors; supervision, L.A.J; project administration, L.A.J.; funding acquisition, L.A.J.

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References

Agarwal, P., Morriseau, T. S., Kereliuk, S. M., Doucette, C. A., Wicklow, B. A., &Dolinsky, V. W. (2018). Maternal obesity, diabetes during pregnancy and epigenetic mechanisms that influence the developmental origins of cardiometabolic disease in the offspring. *Critical Reviews in ClinicalLaboratorySciences*, *55*(2), 71–101. https://doi.org/10.1080/10408363.2017.1422109

Aguirre-Gamboa, R., Gomez-Rueda, H., Martínez-Ledesma, E., Martínez-Torteya, A., Chacolla-Huaringa, R., Rodriguez-Barrientos, A., Tamez-Peña, J. G., &Treviño, V. (2013). SurvExpress: An Online Biomarker Validation Tool and Database for Cancer Gene Expression Data Using Survival Analysis. *PLoS ONE*. https://doi.org/10.1371/journal.pone.0074250

Alex, A. B., Pal, S. K., & Agarwal, N. (2016). CYP17 inhibitors in prostate cancer: Latest evidence and clinical potential. In *Therapeutic Advances in Medical Oncology* (Vol. 8, Issue 4, pp. 267–275). SAGE Publications Inc. https://doi.org/10.1177/1758834016642370

Altobelli, G., Bogdarina, I. G., Stupka, E., Clark, A. J. L., & Langley-Evans, S. (2013). Genome-wide methylation and gene expression changes in newborn rats following maternal protein restriction and reversal by folic acid. *PloS One*, *8*(12), e82989. https://doi.org/10.1371/journal.pone.0082989

Angebault, C., Guichet, P. O., Talmat-Amar, Y., Charif, M., Gerber, S., Fares-Taie, L., Gueguen, N., Halloy, F., Moore, D., Amati-Bonneau, P., Manes, G., Hebrard, M., Bocquet, B., Quiles, M., Piro-Mégy, C., Teigell, M., Delettre, C., Rossel, M., Meunier, I., … Lenaers, G. (2015). Recessive mutations in RTN4IP1 cause isolated and syndromic optic neuropathies. *American Journal of Human Genetics*. https://doi.org/10.1016/j.ajhg.2015.09.012

Arnold, C., Konkel, A., Fischer, R., &Schunck, W. H. (2010). Cytochrome p450-dependent metabolism of ω-6 and ω-3 long-chain polyunsaturated fatty acids. In *Pharmacological Reports* (Vol. 62, Issue 3, pp. 536–547). Elsevier B.V. https://doi.org/10.1016/S1734-1140(10)70311-X

Bai, Y., Wang, G., Fu, W., Lu, Y., Wei, W., Chen, W., Wu, X., Meng, H., Feng, Y., Liu, Y., Li, G., Wang, S., Wang, K., Dai, J., Li, H., Li, M., Huang, J., Li, Y., Wei, S., … Guo, H. (2019). Circulating essential metals and lung cancer: Risk assessment and potential molecular effects. *EnvironmentInternational*, *127*, 685–693. https://doi.org/10.1016/j.envint.2019.04.021

Bautista, C. J., Rodríguez-González, G. L., Morales, A., Lomas-Soria, C., Cruz-Pérez, F., Reyes-Castro, L. A., &Zambrano, E. (2017). Maternal obesity in the rat impairs male offspring aging of the testicular antioxidant defence system. *Reproduction, Fertility, and Development*. https://doi.org/10.1071/RD16277

Berglund, L., Björling, E., Oksvold, P., Fagerberg, L., Asplund, A., Szigyarto, C. A. K., Persson, A., Ottosson, J., Wernérus, H., Nilsson, P., Lundberg, E., Sivertsson, Å., Navani, S., Wester, K., Kampf, C., Hobert, S., Pontén, F., &Uhlén, M. (2008). A genecentric human protein atlas for expression profiles based on antibodies. In *Molecular and Cellular Proteomics*. https://doi.org/10.1074/mcp.R800013-MCP200

Bruikman, C. S., Zhang, H., Kemper, A. M., Van Gils, J. M., &Majerciak, V. (2019). Netrin Family: Role for Protein Isoforms in Cancer. In *Journal of Nucleic Acids*. https://doi.org/10.1155/2019/3947123

Cerami, E., Gao, J., Dogrusoz, U., Gross, B. E., Sumer, S. O., Aksoy, B. A., Jacobsen, A., Byrne, C. J., Heuer, M. L., Larsson, E., Antipin, Y., Reva, B., Goldberg, A. P., Sander, C., & Schultz, N. (2012). The cBio Cancer Genomics Portal: An open platform for exploring multidimensional cancer genomics data. *Cancer Discovery*. https://doi.org/10.1158/2159-8290.CD-12-0095

Chandrashekar, D. S., Bashel, B., Balasubramanya, S. A. H., Creighton, C. J., Ponce-Rodriguez, I., Chakravarthi, B. V. S. K., &Varambally, S. (2017). UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. *Neoplasia (United States)*, *19*(8), 649–658. https://doi.org/10.1016/j.neo.2017.05.002

Chang, G. W. M., & Kam, P. C. A. (1999). The physiological and pharmacological roles of cytochrome P450 isoenzymes. *Anaesthesia*. https://doi.org/10.1046/j.1365-2044.1999.00602.x

Cheong, A., Zhang, X., Cheung, Y.-Y., Tang, W.-Y., Chen, J., Ye, S.-H., Medvedovic, M., Leung, Y.-K., Prins, G. S., & Ho, S.-M. (2016). DNA methylome changes by estradiol benzoate and bisphenol A links early-life environmental exposures to prostate cancer risk. *Epigenetics*, *11*(9), 674–689. https://doi.org/10.1080/15592294.2016.1208891

Clarke, D. J. B., Kuleshov, M. V., Schilder, B. M., Torre, D., Duffy, M. E., Keenan, A. B., Lachmann, A., Feldmann, A. S., Gundersen, G. W., Silverstein, M. C., Wang, Z., &Ma'Ayan, A. (2018). EXpression2Kinases (X2K) Web: Linking expression signatures to upstream cell signaling networks.

NucleicAcidsResearch. https://doi.org/10.1093/nar/gky458

Colombelli, K. T., Santos, S. A. A., Camargo, A. C. L., Constantino, F. B., Barquilha, C. N., Rinaldi, J. C., Felisbino, S. L., &Justulin, L. A. (2017). Impairment of microvascular angiogenesis is associated with delay in prostatic development in rat offspring of maternal protein malnutrition. *General and Comparative Endocrinology*, *246*, 258–269. https://doi.org/10.1016/j.ygcen.2016.12.016

Cyrus, S., Burkardt, D., Weaver, D. D., & Gibson, W. T. (2019). PRC2-complex related dysfunction in overgrowth syndromes: A review of EZH2, EED, and SUZ12 and their syndromic phenotypes. *American Journal of Medical Genetics, Part C: Seminars in Medical Genetics*, *181*(4), 519–531. https://doi.org/10.1002/ajmg.c.31754

Damiani, D., Goffinet, A. M., Alberts, A., &Tissir, F. (2016). Lack of Diaph3 relaxes the spindle checkpoint causing the loss of neural progenitors. *Nature Communications*. https://doi.org/10.1038/ncomms13509

Daniel, Z., Swali, A., Emes, R., & Langley-Evans, S. C. (2016). The effect of maternal undernutrition on the rat placental transcriptome: protein restriction up-regulates cholesterol transport. *Genes & Nutrition*, *11*(1), 27. https://doi.org/10.1186/s12263-016-0541-3

De Alexandre, R. B., Horvath, A. D., Szarek, E., Manning, A. D., Leal, L. F., Kardauke, F., Epstein, J. A., Carraro, D. M., Soares, F. A., Apanasovich, T. V., Stratakis, C. A., &Faucz, F. R. (2015). Phosphodiesterase sequence variants may predispose to prostate cancer. *Endocrine-Related Cancer*. https://doi.org/10.1530/ERC-15-0134

de Mello Santos, T., Cavariani, M. M., Pereira, D. N., Schimming, B. C., Chuffa, L. G. de A., &Domeniconi, R. F. (2019). Maternal Protein Restriction Modulates Angiogenesis and AQP9 Expression Leading to a Delay in Postnatal Epididymal Development in Rat. *Cells*, *8*(9), 1094. https://doi.org/10.3390/cells8091094

Dulos, J., Van Der Vleuten, M. A. J., Kavelaars, A., Heijnen, C. J., & Boots, A. M. (2005). CYP7B expression and activity in fibroblast-like synoviocytes from patients with rheumatoid arthritis: Regulation by proinflammatory cytokines. *Arthritis and Rheumatism*. https://doi.org/10.1002/art.20950

Dupont, S., Krust, A., Gansmuller, A., Dierich, A., Chambon, P., & Mark, M. (2000). Effect of single and compound knockouts of estrogen receptors alpha (ERalpha) and beta (ERbeta) on mouse reproductive phenotypes. *Development (Cambridge, England)*.

Gajjar, K., Martin-Hirsch, P. L., & Martin, F. L. (2012). CYP1B1 and hormone-induced cancer. In *Cancer Letters* (Vol. 324, Issue 1, pp. 13–30). Elsevier. https://doi.org/10.1016/j.canlet.2012.04.021

Gao, J., Aksoy, B. A., Dogrusoz, U., Dresdner, G., Gross, B., Sumer, S. O., Sun, Y., Jacobsen, A., Sinha, R., Larsson, E., Cerami, E., Sander, C., & Schultz, N. (2013). Integrative Analysis of Complex Cancer

693 Genomics and Clinical Profiles Using the cBioPortal. *Science Signaling*, *6*(269), pl1–pl1.
694 https://doi.org/10.1126/scisignal.2004088

695 Godfrey, K. M., & Barker, D. J. P. (2000). Fetal nutrition and adult disease. *American Journal of Clinical*
696 *Nutrition*, *71*(5 SUPPL.).

697 Goyal, D., Limesand, S. W., & Goyal, R. (2019). Epigenetic responses and the developmental origins of
698 health and disease. In *Journal of Endocrinology*. https://doi.org/10.1530/JOE-19-0009

699 Han, X. H., Jin, Y. R., Seto, M., & Yoon, J. K. (2011). A WNT/β-catenin signaling activator, R-spondin,
700 plays positive regulatory roles during skeletal myogenesis. *Journal of Biological Chemistry*, *286*(12),
701 10649–10659. https://doi.org/10.1074/jbc.M110.169391

702 Han, X., &Liehr, J. G. (1995). Microsome-mediated 8-hydroxylation of guanine bases of DNA by steroid
703 estrogens: Correlation of DNA damage by free radicals with metabolic activation to quinones.
704 *Carcinogenesis*. https://doi.org/10.1093/carcin/16.10.2571

705 Hao, W., Yu, M., Lin, J., Liu, B., Xing, H., Yang, J., Sun, D., Chen, F., Jiang, M., Tang, C., Zhang, X.,
706 Zhao, Y., & Zhu, Y. (2020). The pan-cancer landscape of netrin family reveals potential oncogenic
707 biomarkers. *Scientific Reports*. https://doi.org/10.1038/s41598-020-62117-5

708 Hayes, C. L., Spink, D. C., Spink, B. C., Cao, J. Q., Walker, N. J., & Sutter, T. R. (1996). 17β-Estradiol
709 hydroxylation catalyzed by human cytochrome P450 1B1. *Proceedings of the National Academy of*
710 *Sciences of the United States of America*. https://doi.org/10.1073/pnas.93.18.9776

711 Hrycay, E. G., &Bandiera, S. M. (2015). Involvement of Cytochrome P450 in Reactive Oxygen Species
712 Formation and Cancer. In *Advances in Pharmacology* (Vol. 74, pp. 35–84). Academic Press Inc.
713 https://doi.org/10.1016/bs.apha.2015.03.003

714 Jakobsson, J., Karypidis, H., Johansson, J. E., Roh, H. K., Rane, A., &Ekström, L. (2004). A functional C-
715 G polymorphism in the CYP7B1 promoter region and its different distribution in Orientals and
716 Caucasians. *Pharmacogenomics Journal*. https://doi.org/10.1038/sj.tpj.6500236

717 Jiang, H., Qi, L., Wang, F., Sun, Z., Huang, Z., & Xi, Q. (2015). Decreased semaphorin 3A expression is
718 associated with a poor prognosis in patients with epithelial ovarian carcinoma. *International Journal*
719 *of Molecular Medicine*. https://doi.org/10.3892/ijmm.2015.2142

720 Kuleshov, M. V., Jones, M. R., Rouillard, A. D., Fernandez, N. F., Duan, Q., Wang, Z., Koplev, S., Jenkins,
721 S. L., Jagodnik, K. M., Lachmann, A., McDermott, M. G., Monteiro, C. D., Gundersen, G. W.,
722 &Ma'ayan, A. (2016). Enrichr: a comprehensive gene set enrichment analysis web server 2016
723 update. *Nucleic Acids Research*, *44*(W1), W90–W97. https://doi.org/10.1093/nar/gkw377

724 Langley-Evans, S. (2019). Nutrigenetics and the early life origins of health and disease. In *Principles of*
725 *Nutrigenetics and Nutrigenomics: Fundamentals of Individualized Nutrition*.
726 https://doi.org/10.1016/B978-0-12-804572-5.00015-X

Langley-Evans, S. C., & Sculley, D. V. (2006). The association between birthweight and longevity in the rat is complex and modulated by maternal protein intake during fetal life. *FEBS Letters*, *580*(17), 4150–4153. https://doi.org/10.1016/j.febslet.2006.06.062

Li, C., Long, B., Qin, X., Li, W., & Zhou, Y. (2015). Cytochrome P1B1 (CYP1B1) polymorphisms and cancer risk: A meta-analysis of 52 studies. In *Toxicology* (Vol. 327, pp. 77–86). Elsevier Ireland Ltd. https://doi.org/10.1016/j.tox.2014.11.007

Livak, K. J., &Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. *Methods*, *25*(4), 402–408. https://doi.org/10.1006/meth.2001.1262

Maksymchuk, O., &Kashuba, V. (2019). Dietary lipids and environmental xenobiotics as risk factors for prostate cancer: The role of cytochrome P450. *Pharmacological Reports*. https://doi.org/10.1016/j.pharep.2019.04.011

Manikandan, P., &Nagini, S. (2017). Cytochrome P450 Structure, Function and Clinical Significance: A Review. *Current Drug Targets*. https://doi.org/10.2174/1389450118666170125144557

Martin, C., Ross, M., Chapman, K. E., Andrew, R., Bollina, P., Seckl, J. R., & Habib, F. K. (2004). CYP7B generates a selective estrogen receptor β agonist in human prostate. *Journal of Clinical Endocrinology and Metabolism*. https://doi.org/10.1210/jc.2003-031847

Martin, F. L., Patel, I. I., Sozeri, O., Singh, P. B., Ragavan, N., Nicholson, C. M., Frei, E., Meinl, W., Glatt, H., Phillips, D. H., &Arlt, V. M. (2010). Constitutive expression of bioactivating enzymes in normal human prostate suggests a capability to activate pro-carcinogens to DNA-damaging metabolites. *Prostate*. https://doi.org/10.1002/pros.21194

Mazor, M., Kawano, Y., Zhu, H., Waxman, J., &Kypta, R. M. (2004). Inhibition of glycogen synthase kinase-3 represses androgen receptor activity and prostate cancer cell growth. *Oncogene*, *23*(47), 7882–7892. https://doi.org/10.1038/sj.onc.1208068

McDonnell, PharmD, BCOP, A. M., & Dang, PharmD, BCPS, C. H. (2013). Basic Review of the Cytochrome P450 System. *Journal of the Advanced Practitioner in Oncology*, *4*(4). https://doi.org/10.6004/jadpro.2013.4.4.7

Metsalu, T., &Vilo, J. (2015). ClustVis: A web tool for visualizing clustering of multivariate data using Principal Component Analysis and heatmap. *Nucleic Acids Research*, *43*(W1), W566–W570. https://doi.org/10.1093/nar/gkv468

Mirosevich, J., Gao, N., Gupta, A., Shappell, S. B., Jove, R., &Matusik, R. J. (2006). Expression and role of Foxa proteins in prostate cancer. *The Prostate*, *66*(10), 1013–1028. https://doi.org/10.1002/pros.20299

Nebert, D. W., & Dalton, T. P. (2006). The role of cytochrome P450 enzymes in endogenous signalling

pathways and environmental carcinogenesis. In *Nature Reviews Cancer* (Vol. 6, Issue 12, pp. 947–960). Nat Rev Cancer. https://doi.org/10.1038/nrc2015

Nishimura, M., Yaguti, H., Yoshitsugu, H., Naito, S., & Satoh, T. (2003). Tissue distribution of mRNA expression of human cytochrome P450 isoforms assessed by high-sensitivity real-time reverse transcription PCR. *YakugakuZasshi*, *123*(5), 369–375. https://doi.org/10.1248/yakushi.123.369

Obligacion, R. (2006). Drug-Metabolizing Enzymes and Transporters: Expression in the Human Prostate and Roles in Prostate Drug Disposition. *Journal of Andrology*, *27*(2), 138–150. https://doi.org/10.2164/jandrol.05113

Olsson, M., Gustafsson, O., Skogastierna, C., Tolf, A., Rietz, B. Du, Morfin, R., Rane, A., Ekström, L., Du Rietz, B., Morfin, R., Rane, A., &Ekström, L. (2007). *Regulation and expression of human CYP7B1 in prostate: Overexpression of CYP7B1 during progression of prostatic adenocarcinoma*. *67*(13), 1439–1446. https://doi.org/10.1002/pros.20630

Omoto, Y., Lathe, R., Warner, M., & Gustafsson, J. Å. (2005). Early onset of puberty and early ovarian failure in CYP7B1 knockout mice. *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.0500198102

Pak, T. R., Chung, W. C. J., Lund, T. D., Hinds, L. R., Clay, C. M., &Handa, R. J. (2005). The androgen metabolite, 5α-androstane-3β, 17β-diol, is a potent modulator of estrogen receptor-β1-mediated gene transcription in neuronal cells. *Endocrinology*. https://doi.org/10.1210/en.2004-0871

Papavassiliou, K. A., &Papavassiliou, A. G. (2016). Transcription Factor Drug Targets. *Journal of Cellular Biochemistry*, *117*(12), 2693–2696. https://doi.org/10.1002/jcb.25605

Peng, H. M., Porter, T. D., Ding, X., & Coon, M. J. (1991). Differences in the developmental expression of rabbit cytochromes P-450 2E1 and 2E2. *Molecular Pharmacology*.

Pinho, C. F., Ribeiro, M. a., Rinaldi, J. C., Felisbino, S. L., Pinheiro, P. F., Domeniconi, R. F., Fochi, R. A., Boer, P. A. P. a., & Scarano, W. R. (2014). Gestational protein restriction delays prostate morphogenesis in male rats. *Reproduction, Fertility and Development*, *26*(7), 967–973. https://doi.org/10.1071/RD13132

Prins, G. S., & Putz, O. (2008). Molecular signaling pathways that regulate prostate gland development. In *Differentiation* (Vol. 76, Issue 6, pp. 641–659). https://doi.org/10.1111/j.1432-0436.2008.00277.x

Prins, G. S., Ye, S.-H., Birch, L., Zhang, X., Cheong, A., Lin, H., Calderon-Gierszal, E., Groen, J., Hu, W.-Y., Ho, S.-M., & van Breemen, R. B. (2017). Prostate Cancer Risk and DNA Methylation Signatures in Aging Rats following Developmental BPA Exposure: A Dose–Response Analysis. *Environmental Health Perspectives*, *125*(7), 077007. https://doi.org/10.1289/EHP1050

Puchtler, H., Waldrop, F. S., Meloan, S. N., Terry, M. S., & Conner, H. M. (1970). Methacarn (methanol-Carnoy) fixation. Practical and theoretical considerations. *Histochemie. Histochemistry. Histochimie*,

21(2), 97–116. http://www.ncbi.nlm.nih.gov/pubmed/4907154

Pylayeva-Gupta, Y., Grabocka, E., & Bar-Sagi, D. (2011). RAS oncogenes: Weaving a tumorigenic web. In *Nature Reviews Cancer* (Vol. 11, Issue 11, pp. 761–774). Nat Rev Cancer. https://doi.org/10.1038/nrc3106

Qasem, R. J., Yablonski, E., Li, J., Tang, H. M., Pontiggia, L., & D'mello, A. P. (2012). Elucidation of thrifty features in adult rats exposed to protein restriction during gestation and lactation. *Physiology and Behavior*. https://doi.org/10.1016/j.physbeh.2011.12.010

Richter, A., Valdimarsdottir, L., Hrafnkelsdottir, H. E., Runarsson, J. F., Omarsdottir, A. R., Ward-van Oostwaard, D., Mummery, C., &Valdimarsdottir, G. (2014). BMP4 promotes EMT and mesodermal commitment in human embryonic stem cells via SLUG and MSX2. *StemCells (Dayton, Ohio)*, *32*(3), 636–648. https://doi.org/10.1002/stem.1592

Rinaldi, J. C., Justulin, L. a., Lacorte, L. M., Sarobo, C., Boer, P. a., Scarano, W. R., &Felisbino, S. L. (2013). Implications of intrauterine protein malnutrition on prostate growth, maturation and aging. *Life Sciences*, *92*(13), 763–774. https://doi.org/10.1016/j.lfs.2013.02.007

Santos, S. A. A., Camargo, A. C., Constantino, F. B., Colombelli, K. T., Mani, F., Rinaldi, J. C., Franco, S., Portela, L. M. F., Duran, B. O. S., Scarano, W. R., Hinton, B. T., Felisbino, S. L., &Justulin, L. A. (2019). Maternal Low-Protein Diet Impairs Prostate Growth in Young Rat Offspring and Induces Prostate Carcinogenesis with Aging. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, *74*(6), 751–759. https://doi.org/10.1093/gerona/gly118

Scarano, W. R., Bedrat, A., Alonso-Costa, L. G., Aquino, A. M., Fantinatti, B., Justulin, L. A., Barbisan, L. F., Freire, P. P., Flaws, J. A., & Bernardo, L. (2019). Exposure to an environmentally relevant phthalate mixture during prostate development induces microRNA upregulation and transcriptome modulation in rats. *Toxicological Sciences: An Official Journal of the Society of Toxicology*. https://doi.org/10.1093/toxsci/kfz141

Simicevic, J., &Deplancke, B. (2017). Transcription factor proteomics—Tools, applications, and challenges. In *Proteomics*. https://doi.org/10.1002/pmic.201600317

Song, Z., & Shi, Q. (2017). The interaction of PPARα and CYP7B1 with ERα,β impacted the occurrence and development of intrahepatic cholestasis in pregnant rats. *Reproductive Sciences*. https://doi.org/10.1177/1933719116667223

Spink, D. C., Spink, B. C., Cao, J. Q., Gierthy, J. F., Hayes, C. L., Ying, L., & Sutter, T. R. (1997). Induction of cytochrome P450 1B1 and catechol estrogen metabolismin ACHN human renal adrenocarcinoma cells. *Journal of Steroid Biochemistry and Molecular Biology*. https://doi.org/10.1016/S0960-0760(97)00024-1

Sutter, T. R., Tang, Y. M., Hayes, C. L., Wo, Y. Y., Jabs, E. W., Li, X., Yin, H., Cody, C. W., & Greenlee,

W. F. (1994). Complete cDNA sequence of a human dioxin-inducible mRNA identifies a new gene subfamily of cytochrome P450 that maps to chromosome 2. *The Journal of Biological Chemistry*, *269*(18), 13092–13099. http://www.ncbi.nlm.nih.gov/pubmed/8175734

Suzuki, K. (2018). The developing world of DOHaD. *Journal of Developmental Origins of Health and Disease*, *9*(3), 266–269. https://doi.org/10.1017/S2040174417000691

Tang, W., &Norlin, M. (2006). Regulation of steroid hydroxylase CYP7B1 by androgens and estrogens in prostate cancer LNCaP cells. *Biochemical and Biophysical Research Communications*. https://doi.org/10.1016/j.bbrc.2006.03.175

Tsuchiya, Y., Nakajima, M., & Yokoi, T. (2005). Cytochrome P450-mediated metabolism of estrogens and its regulation in human. *Cancer Letters*. https://doi.org/10.1016/j.canlet.2004.10.007

Vladar, E. K., Lee, Y. L., Stearns, T., & Axelrod, J. D. (2015). *HHS Public Access*. *6*(2), 37–54. https://doi.org/10.1016/bs.mcb.2015.01.016.Observing

Wang, G., Wang, J., &Sadar, M. D. (2008). Crosstalk between the Androgen Receptor and -Catenin in Castrate-Resistant Prostate Cancer. *Cancer Research*, *68*(23), 9918–9927. https://doi.org/10.1158/0008-5472.CAN-08-1718

Weihua, Z., Lathe, R., Warner, M., & Gustafsson, J. Å. (2002). An endocrine pathway in the prostate, ERβ, AR, 5α-androstane-3β, 17β-diol, and CYP7B1, regulates prostate growth. *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.162477299

Welsh, M., Saunders, P. T. K., Fisken, M., Scott, H. M., Hutchison, G. R., Smith, L. B., & Sharpe, R. M. (2008). Identification in rats of a programming window for reproductive tract masculinization, disruption of which leads to hypospadias and cryptorchidism. *Journal of Clinical Investigation*, *118*(4), 1479–1490. https://doi.org/10.1172/JCI34241

Xu, G., Zhang, L., Ma, A., Qian, Y., Ding, Q., Liu, Y., Wang, B., Yang, Z., & Liu, Y. (2015). SIP1 is a downstream effector of GADD45G in senescence induction and growth inhibition of liver tumor cells. *Oncotarget*. https://doi.org/10.18632/oncotarget.5602

Zambrano, E., Rodríguez-González, G. L., Guzmán, C., García-Becerra, R., Boeck, L., Díaz, L., Menjivar, M., Larrea, F., &Nathanielsz, P. W. (2005). A maternal low protein diet during pregnancy and lactation in the rat impairs male reproductive development. *The Journal of Physiology*, *563*(Pt 1), 275–284. https://doi.org/10.1113/jphysiol.2004.078543

Zhang, S., Li, L. hu, Qiao, H. mei, Yang, X., Chen, L., & Luo, X. hui. (2019). Regulation of the Antioxidant Response by MyoD Transcriptional Coactivator in Castration-resistant Prostate Cancer Cells. *Urology*, *123*, 296.e9-296.e18. https://doi.org/10.1016/j.urology.2018.04.028

Zhang, Y., Gaikwad, N. W., Olson, K., Zahid, M., Cavalieri, E. L., &Rogan, E. G. (2007). Cytochrome

P450 isoforms catalyze formation of catechol estrogen quinones that react with DNA. *Metabolism: Clinical and Experimental*. https://doi.org/10.1016/j.metabol.2007.03.001

Zhu, B. T., &Conney, A. H. (1998). Functional role of estrogen metabolism in target cells: Review and perspectives. In *Carcinogenesis*. https://doi.org/10.1093/carcin/19.1.1

Supplementary Material

Transcriptomic landscape reveals molecular signaling pathways related to prostate cancer risk in maternal malnourished offspring rats

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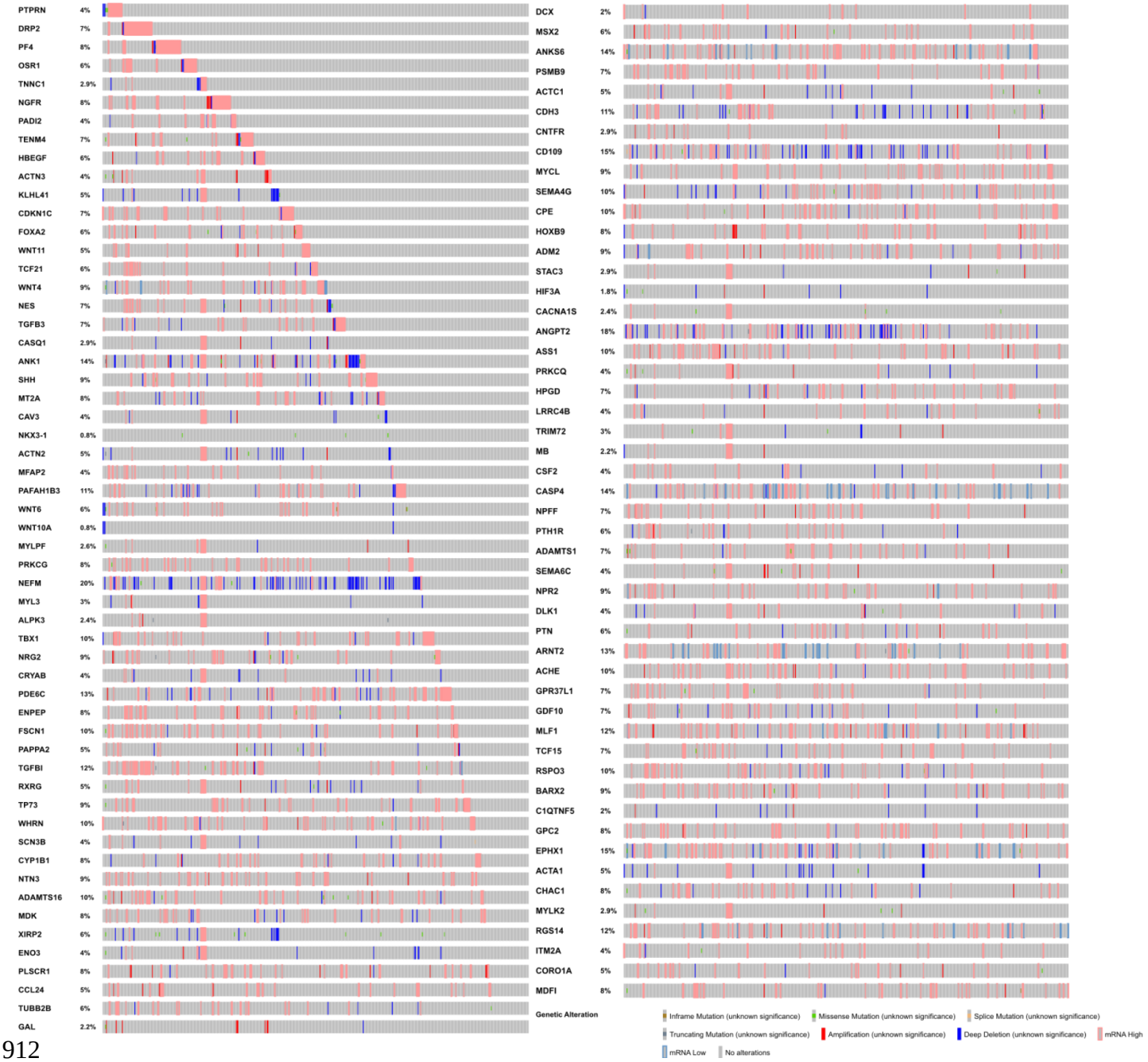
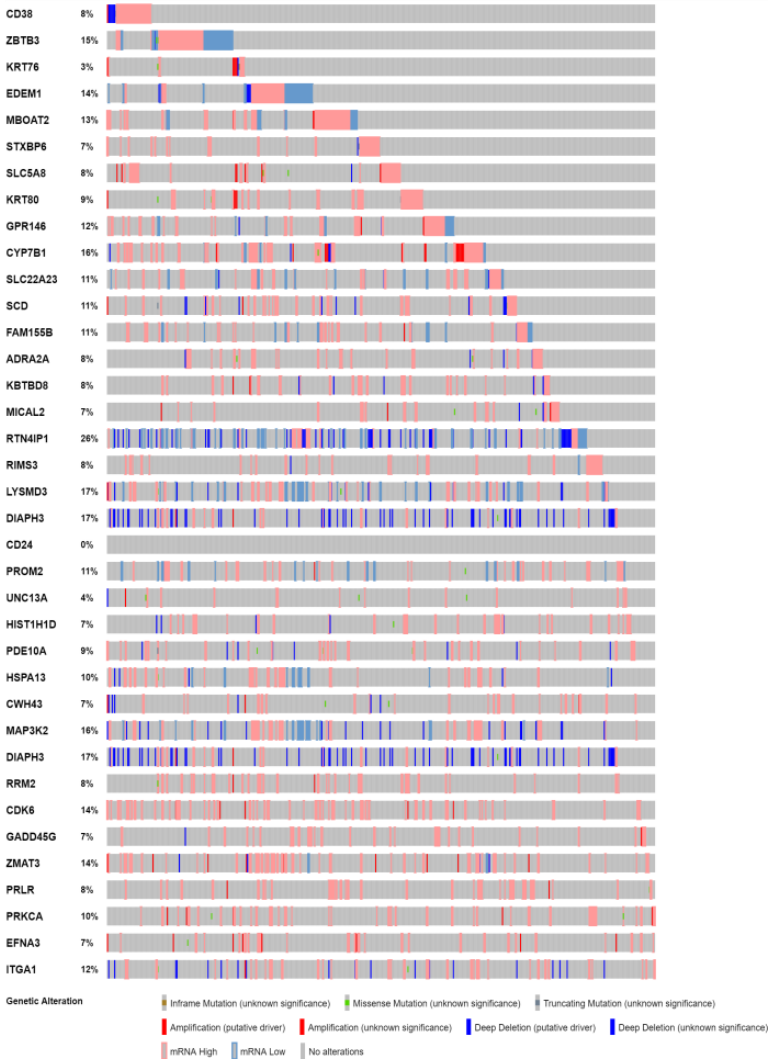
Supplemtarys figure

A



B



Supplementary Figure 1. Genetic alterations in DEGs. Oncoprint data represent Prostate Adenocarcinoma (TCGA, Provisional) samples published in cBioPortal. The samples consulted represent tumors enriched with 106 upregulated **(A)** and 38 downregulated **(B)** genes, the set of genes was altered 98% (483/498) and 93% (458/498) of queried patients/samples, respectively. The most common type of changes observed in both sets were high mRNA expression, amplification and deletion.

Supplementary Figure 2. Expression genes across PRAD- TCGA. Heatmap showing expression profile of 106 upregulated and 38 downregulated genes from Kobas analyses acros PRAD- TCGA, generated by UALCAN.

Supplemtarys table

Supplementary Table 1. Composition of control diet and low protein diet.

Ingredients	Control diet (17% of protein g/kg)	Low protein diet (6 % of proteing/kg)
Casein (84% of protein)	202	71.5
Starch	397	480
Dextrin	130.5	159
Sucrose	100	121
L-cysteine	3	1
Fiber pH 101 or pH 102 (microcellulose)	50	50
Soy oil	70	70
Mixture of salts AIN93G *	35	35
Mixture of vitamins AIN93G *	10	10
Choline Hydrochloride or Choline Bitartrate	2.5	2.5

* To know the detailed composition of the salt and vitamin mix, see REEVES et al. [51]

Supplementary Table 2. Differentially expressed genes (DEGs)- file excel

Supplementary Table 3. Enrichment terms of upregulated and downregulated DEGs (Excel)

Supplementary Table 4. Bibliographic of four protein-coding genes by PubMed.

Gene	Function	Reference
Cyp1b1	Able to metabolize steroid hormones/ Cyp1b1 have been implicated directly in the regulation of embryonic development.	D.Choudhary, D et. al, 2003
	CYP1B1 is necessary for normal embryonic or fetal tissue development, the appearance of these two mutations could result in developmental abnormalities.	Jansson, I et. al, 2001
	Encodes enzymes that convert 17B-estradiol in 4 hydroxyestradiol, a pro-carcinogenic molecule that triggers the activation of angiogenic processes. Suppression associated with PCa, responsible to activates caspases.	Chang, et. al., 2017
Rspo3	Wnt and R-spondin gene expression in the developing male mouse lower urogenital tract.	Mehta et. al, 2011
	R-spondins are BMP receptor antagonists in Xenopus early embryonic development.	Lee et. al, 2020
	Responsible for regulating Wnt signaling pathways, b-cathetins and G-protein coupled pathways. In the prostate, it could act as a prognostic and invasiveness biomarker, responsible for contributing to the gland angiogenesis.	Mesci et. al, 2019
Cyp7b1	Involved in the metabolism of various steroids, affecting estrogen pathways and androgen signaling.	Maksymchuk et. al, 2019
	CYP7B1 represents the major pathway for inactivating 3betaAdiol in the prostate, we suggest that ERbeta, 3betaAdiol, and CYP7B1 are the components of a pathway that regulates growth of the rodent ventral prostate.	Pettersson 1, H et.al, 2008
	In the reproductive tract, the enzyme metabolizes androgens that antagonize estrogen action; mice without CYP7B1 have abnormal prostates and ovaries.	Stiles AR et. al, 2009
Gadd45g	Prostate cancer: JunD, Gadd45a and Gadd45g as therapeutic targets.	Dan A. Liebermann & Barbara Hoffman. 2011
	Gadd45g expression during mouse embryonic development/ Related to stress signaling in response to physiological and environmental stress, including oncogenic stress, which can result in cell cycle arrest, DNA repair, cell survival, senescence and apoptosis.	Kaufmann, et.al. 2011
	Gadd45g is essential for primary sex determination, male fertility and testis development.	Johnen H, et. al. 2013

Supplementary Table 5. Functional enrichment terms by Enrichr tool.

Database	Term	P-value	Genes
Elsevier Pathway collection	Metabolism of Estrogens and Androgens	1.0E-03	CYP1B1
	Prostate Cancer	2.4E-02	GADD45G
DisGeNET	Male infertility	1.0E-03	CYP1B1;GADD45G
	Malignant neoplasm of prostate	1.5E-02	CYP1B1;CYP7B1;GADD45G
	Prostate carcinoma	1.4E-02	CYP1B1;CYP7B1;GADD45G
Disease Pertubation from GEO_up	Prostate cancer DOID-10283 human GSE26910 sample 603	1.4E-03	CYP1B1;RSPO3
BioPlanet_2019	Cytochrome P450 metabolism of endogenous sterols	3.1E-06	CYP1B1;CYP7B1
	Steroid hormone biosynthesis	4.6E-05	CYP1B1;CYP7B1
	Cytochrome P450 pathway	5.5E-05	CYP1B1;CYP7B1
	MicroRNA regulation of DNA damage response	1.4E-02	GADD45G
	Estrogen metabolism	3.4E-03	CYP1B1
KEGG_2019	Steroid hormone biosynthesis	5.3E-05	CYP1B1;CYP7B1
	Transcriptional misregulation in cancer	3.7E-02	GADD45G
	p53 signaling pathway	1.4E-02	GADD45G
GO_MF	Aromatase activity (GO:0070330)	1.2E-03	CYP1B1
GO_BP	Positive regulation of non-canonical Wnt signaling pathway (GO:2000052)	1.6E-03	RSPO3
	Positive regulation of apoptotic process (GO:0043065)	1.4E-03	CYP1B1;GADD45G
	Steroid metabolic process (GO:0008202)	1.4E-04	CYP1B1;CYP7B1
	Regulation of non-canonical Wnt signaling pathway (GO:2000050)	2.2E-03	RSPO3

Significant when p value ≤ 0.05.

Capítulo 3 – Artigo II

Research Paper

Identification of potential molecular pathways involved in prostate carcinogenesis in offspring exposed to maternal malnutrition

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ABSTRACT

The developmental origins of health and disease concept links adult diseases with early-life exposure to inappropriate environmental conditions. Intrauterine and postnatal malnutrition may lead to an increased incidence of type 2 diabetes, obesity, and cardiovascular diseases. Maternal malnutrition (MM) has also been associated with prostate carcinogenesis. However, the molecular mechanisms associated with this condition remain poorly understood. Using a proteomic analysis, we demonstrated that MM changed the levels of proteins associated with growth factors, estrogen signaling, detoxification, and energy metabolism in the prostate of both young and old rats. These animals also showed increased levels of molecular markers of endoplasmic reticulum function and histones. We further performed an *in silico* analysis that identified commonly deregulated proteins in the ventral prostate of old rats submitted to MM with a mouse model and patients with prostate cancer. In conclusion, our results demonstrated that estrogenic signaling pathways, endoplasmic reticulum functions, energy metabolism, and molecular sensors of protein folding and Ca2+ homeostasis, besides histone, and RAS-GTPase family appear to be involved in this process. Knowledge of these factors may raise discussions regarding the role of maternal dietary intervention as a public policy for the lifelong prevention of chronic diseases.

INTRODUCTION

Advances in public health and preventive medicine have resulted in an unprecedented and welcomed number of individuals reaching old age. Despite longevity, there has been observed an increase in older populations affected by chronic diseases that demand specialized and expensive elderly care services. The identification and widespread public awareness of unhealthy modifiable risk factors such as western diet consumption and obesity, sedentary lifestyle, smoking, stress, and insufficient sleep, followed by the adoption of a healthy lifestyle is a feasible, safe, and effective low-cost public policy program to improve the quality of life with aging [1, 2]. Intrauterine and early postnatal life experiences may permanently modulate health trajectories across the lifespan [3–5]. The hypothesis that the intrauterine period of development may modulate offspring postnatal health was initially proposed by David Barker in "Fetal Origin of Adult Diseases" (FOAD), almost 30 years ago [6]. Subsequently, FOAD evolved to consolidate the "Developmental Origins of Health and Disease" (DOHaD) concept by including both pre-conception and early postnatal life as a new window of susceptibility. Recently, epigenetics has become one of the most relevant molecular mechanisms associated with the transgenerational inheritance involved with DOHaD [7].

Despite the difficulty in confirming the impact of maternal and early life adversity on human health, some tragic events in human history were crucial in supporting the DOHaD concept. For example, the "Dutch Hunger Winter" was a period of severe famine in the western part of the Netherlands at the end of World War II and has provided an opportunity to explore the effects of intrauterine malnutrition on subsequent adult health [8]. First published in 1976, the Dutch Hunger Winter Cohort has been explored to confirm the developmental origin of non-communicable chronic diseases, e.g., cardiovascular disease, obesity, type 2 diabetes, schizophrenia, and infertility in the progeny exposed to famine [9–11]. Importantly, epidemiological and experimental studies have also supported the DOHaD concept as a mechanistic framework related to early life carcinogenesis (such as breast and prostate cancer) [12–17]. Among the malignancies affecting men, prostate cancer (PCa) is the second most diagnosed cancer worldwide. In 2018, the Global Cancer Statistics (GLOBOCAN) estimated almost 1.3 million new cases of PCa globally, leading to 359,000 deaths [18]. Although multifactorial etiology, genetic background, ethnicity, and aging are consistently established risk factors for PCa. However, evidence supporting the early origin of PCa is growing. William Gardner (1995) proposed, almost 30 years ago, the "Prenatal origin of PCa" hypothesis [19]. After that, some epidemiological studies have reinforced Gardner's hypothesis on the early life origins of PCa, as diagnosed in older men [14, 20, 21]. These authors proposed that exposure to certain environmental conditions during pregnancy, such as malnutrition or chemical endocrine disruptors, may alter maternal steroid hormone profiles, thereby modifying the offspring's PCa risk throughout life. This effect was consistently observed in African American men, who are at high risk for PCa, and whose mothers have higher levels of estrogen during pregnancy compared to Caucasian women [20].

In one of the few opportunities to explore how exposure to adverse conditions during windows of vulnerability interferes with human PCa, Keinan-Boker et al. [13] demonstrated that Jewish men exposed during early life to famine and stress during the Holocaust were at a higher risk for several types of cancer (including PCa) later in life. Similarly, women severely exposed to famine during the Dutch hunger winter

were at increased risk for breast cancer development [22]. Interestingly, a higher risk for breast cancer was observed for women who were exposed to famine between the ages of 2 and 9 years. Dirx et al. [23] analyzing data from the Netherlands Cohort Study, showed a slight increase in PCa risk among men exposed to famine during adolescence (a critical window of vulnerability for reproductive organs) compared with those men living in northern and southern parts of the Netherlands, who had almost no exposure to famine. These data reinforce the need to explore, in-depth, the potential of early life malnutrition as an environmental risk factor for prostate carcinogenesis across the lifespan.

Emerging experimental studies have been designed to explore the potential of early life exposure to environmental risk factors on prostate carcinogenesis. The intrauterine or neonatal exposure to endocrine disruptors, such as phthalate or bisphenol A, has been associated with the deregulation of critical molecular pathways involved in prostate carcinogenesis in rat offspring [17, 24, 25]. Regarding maternal malnutrition (MM), Santos et al. [16] demonstrated that offspring born from dams fed with a low protein diet (LPD) during gestation and lactation were at high risk of developing prostatic disorders with aging, including carcinoma *in situ* in the ventral prostate (VP) lobe. Overall, it has been proposed that early life exposure to endocrine disruptors or malnutrition may show early deregulation and persistent cellular response to estrogen signaling pathways, probably involving changes in epigenetic markers, such as DNA methylation and the expression of microRNAs, leading to an increased incidence of prostatic disorders with aging [17, 25–27].

Although MM can be identified as a potentially modifiable environmental risk factor for offspring prostate carcinogenesis, there is a lack of information regarding the molecular mechanisms involved in this process. We previously demonstrated, for the first time, that maternal exposure to a low protein diet promotes prostate carcinogenesis in older rat offspring [16]. In the current investigation, we used mass spectrometry to identify, in young and older offspring, changes in the proteomic profile potentially involved in the early life origins of prostate carcinogenesis observed with aging.

RESULTS

Maternal LPD reduced weight gain and imbalance of steroid hormones in male offspring

Offspring body weight was lower in the LPD animals on both postnatal days (PND) 21 and 540 compared to the respective control (CTR) groups (Figure 1A, 1B). The serum steroid hormones estrogen (17β-estradiol) and testosterone (17β-hydroxy-4-androstene-3-one) increased in the GLLP group on PND 21 compared to the CTR group (Figure 1C). However, on PND 540, while estrogen was higher in the GLLP group, testosterone levels decreased compared to the CTR group, leading to an increased estrogen/ testosterone ratio (Figure 1D).

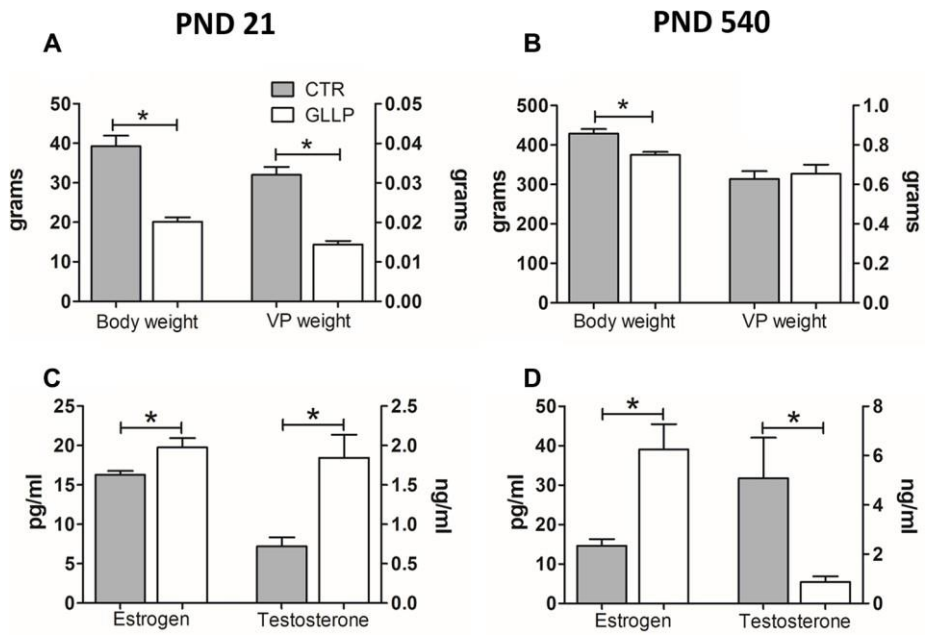

Figure 1. Body weight (A, B) and hormonal levels (C, D) of male offspring on PND 21 and 540. All data are expressed as mean±SD. Asterisks (*) represent statistical differences between experimental groups with $p<0.05$. CTR = control; GLLP = gestational and lactational low protein; PND = postnatal day; VP = ventral prostate.

Early and late effects of maternal LPD on offspring VP

On PND 21, the morphological analyses demonstrated an impairment of prostate growth in the GLLP group, characterized by a smaller prostatic secretory structure, reduced luminal compartment, and increased epithelial and stromal compartments, compared to the CTR group (Figure 2A, 2B). On PND 540, while we did not identify carcinoma in the CTR group (Figure 2C), the histopathological analysis confirmed the presence of carcinoma *in situ* in the animals from the GLLP group selected for a mass spectrometry analysis (Figure 2D).

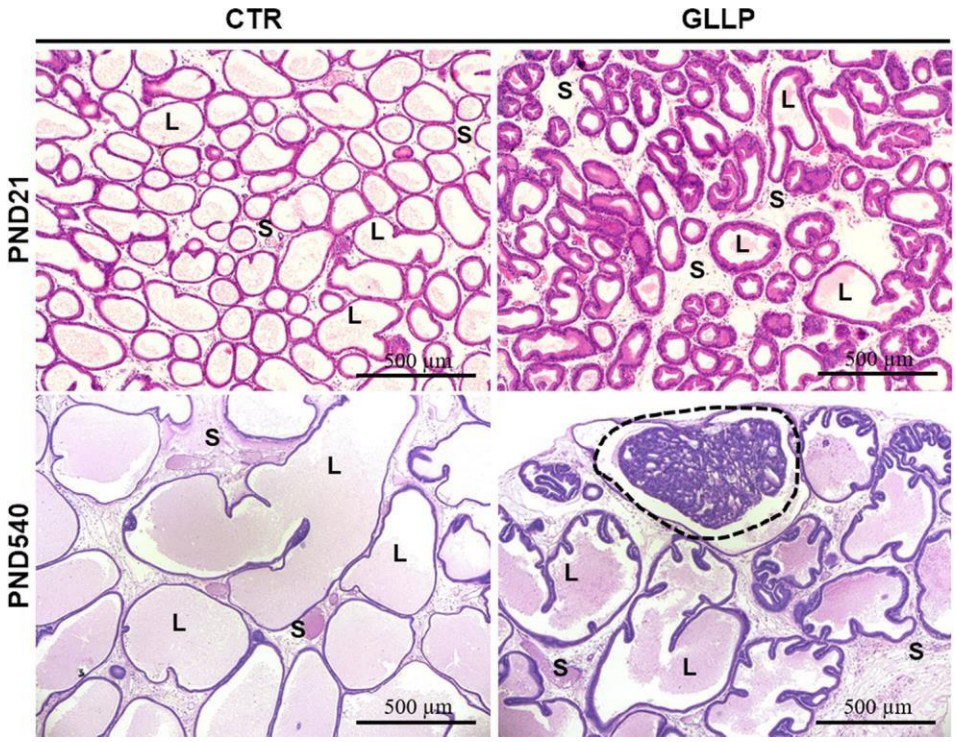

Figure 2. Representative histological sections of the VP lobes from the CTR and GLLP groups on PND 21 and 540, stained with hematoxylin-eosin (HE). Glandular growth in the GLLP group on PND 21 was impaired compared to the CTR. At PND 540, the carcinoma in situ was highlighted by the dashed circle. S = Stroma, L = Lumen, E = Epithelium, Scale bar: 500 µm.

Maternal LPD changed the proteomic profile in the prostate offspring at both ages

Figure 3 shows a total of 256 proteins identified in the VP by MS/MS approach on PND 21. Of these, 158 proteins were significantly differentially expressed in the GLLP group compared to the CTR group, including 138 and 20 proteins that were up- and downregulated, respectively. On PND 540, 366 proteins were significantly differentially expressed in the GLLP group compared to the CTR group, including 135 and 141 proteins that were up- and downregulated, respectively. The complete list of proteins is described in Supplementary File 1.

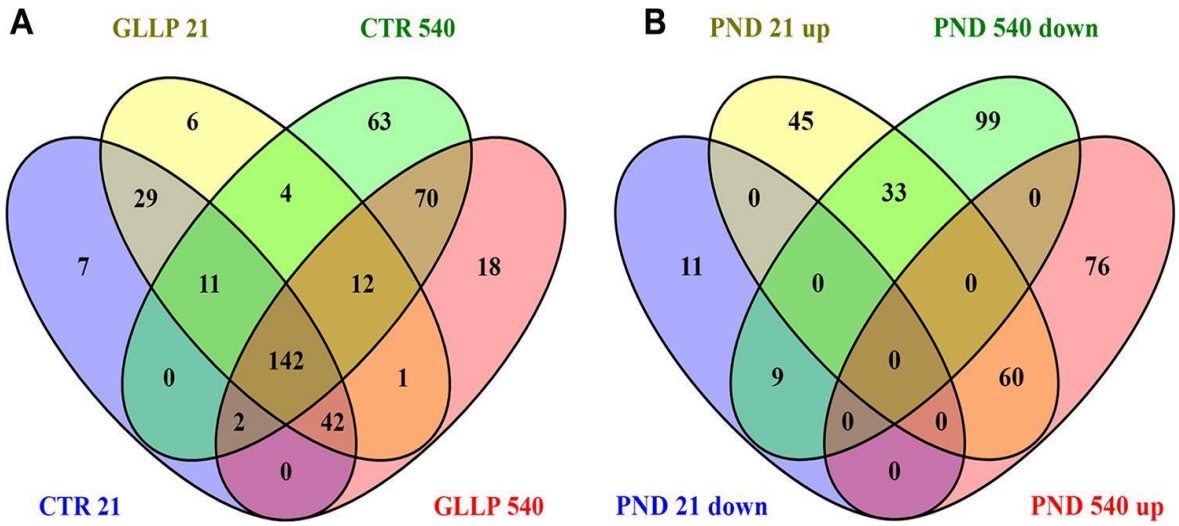

Figure 3. **Venn diagram.** (**A**) Shared proteins between CTR and GLLP groups in PND 21 and 540. (**B**) Shared proteins differentially expressed between the CTR and GLLP groups on PND 21 and 540. CTR = Control; GLLP = Gestational and lactational low protein; PND = Postnatal day; up = upregulated proteins; down = downregulated proteins.

Functional enrichment identified molecular pathways altered by maternal LPD in the offspring prostate

Functional enrichment was performed for the set of downregulated and upregulated proteins separately in both ages. The red and blue bars in Figure 4A demonstrate enriched terms for up and downregulated proteins on PND 21, respectively. Upregulated proteins enriched terms related to protein processing in the endoplasmic reticulum, antigen processing and presentation, metabolism of xenobiotics by cytochrome P450, endocytosis, estrogen signaling pathway, longevity regulating pathway - multiple species, apoptosis signaling pathway, chemical carcinogenesis, spliceosome, glutathione metabolism, arachidonic acid metabolism, drug metabolism - cytochrome P450, and MAPK (mitogen-activated protein kinase) signaling pathways. Downregulated proteins enriched terms related to cell cycle, Hippo signaling pathway, FGF (fibroblast growth factor) signaling pathway, EGF (epidermal growth factor) receptor signaling pathway, cytoskeletal regulation by Rho GTPase, apoptosis, cadherin signaling pathway, gap junction, phosphatidylinositol 3' -kinase (PI3K)-AKT signaling pathway, regulation of actin cytoskeleton, tight junction, focal adhesion, RAP1 (Ras-proximate-1) signaling pathway, adherents junction, and p53 signaling pathway. Using a circus plot analysis, we identified the set of deregulated proteins (up and down) associated with each enriched term on PND 21 (Figure 4B). On PND 540, the upregulated proteins enriched terms related to the metabolism of xenobiotics by cytochrome P450, chemical carcinogenesis, glutathione metabolism, drug metabolism - cytochrome P450, platinum drug resistance, and protein processing in the endoplasmic reticulum. Downregulated protein- enriched terms related to cytoskeletal regulation by Rho-GTPase, phagosome, gap junction, glycolysis/ gluconeogenesis, biosynthesis of amino acids, carbon metabolism, fructose galactose metabolism, regulation of actin cytoskeleton, glycolysis, and cadherin signaling pathway (Figure 5A). Using a circus plot analysis, we identified the set of deregulated proteins (up and down) associated with

each enriched term on PND 540 (Figure 5B). The list of proteins that enriched each molecular term is described in Supplementary File 2.

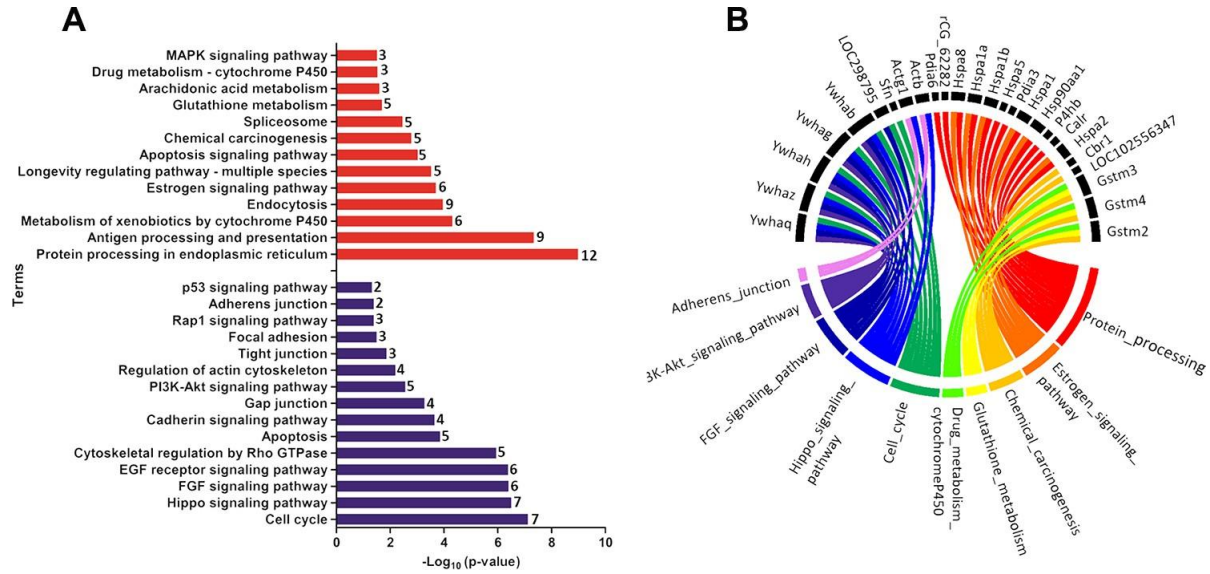

Figure 4. (**A**) Ontological enrichment of upregulated (red) and downregulated (blue) proteins on PND 21 by KOBAs 3.0. All data were expressed as -Log10 (p-value). (**B**) Circus plot graphic identifying the top 10 enriched terms and the DEP associated with each term. The numbers in front of the bars mean the number of proteins that enriched each term.

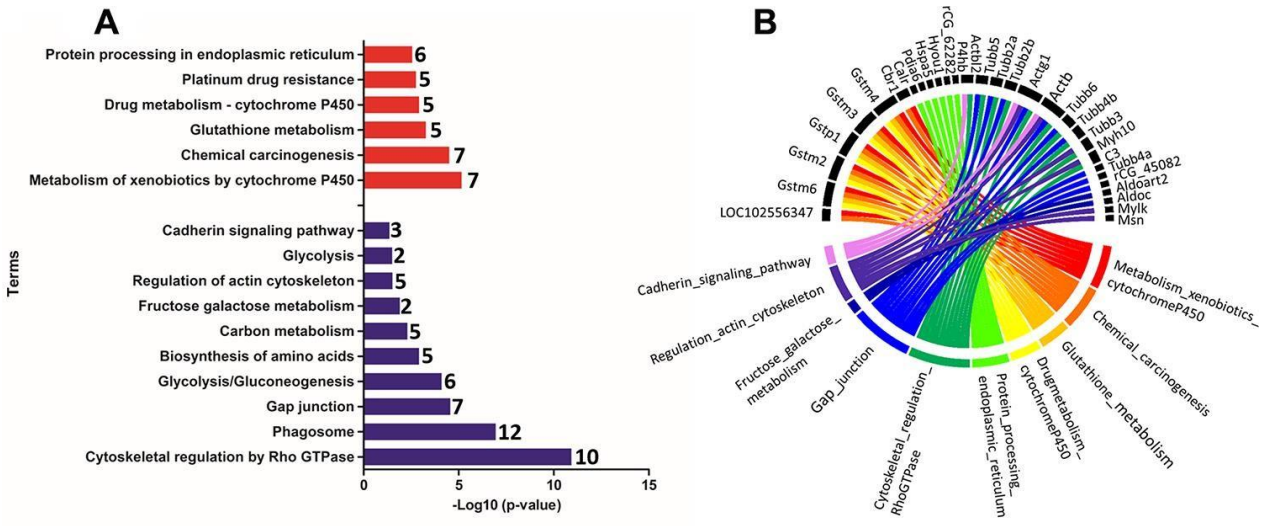

Figure 5. (**A**) Ontological enrichment of upregulated (red) and downregulated (blue) proteins on PND 540 using the KOBAs 3.0 tool. All data were expressed as -Log10 (p-value). (**B**) The Circus plot graphic identifying the top 10 enriched terms and the DEP associated with each term.

The enrichment analysis from Ligand Perturbation UP/DOWN database showed that upregulated proteins (in both PND 21 and 540) are associated with hormonal treatment, especially testosterone and estrogen (Figure 6A, 6B). The set of downregulated proteins for both ages also enriched terms related to exposure to testosterone and estrogen (Figure 7A, 7B). Overall, these results highlighted the involvement of a hormonal imbalance on maternal LPD-induced prostate disorders in offspring.

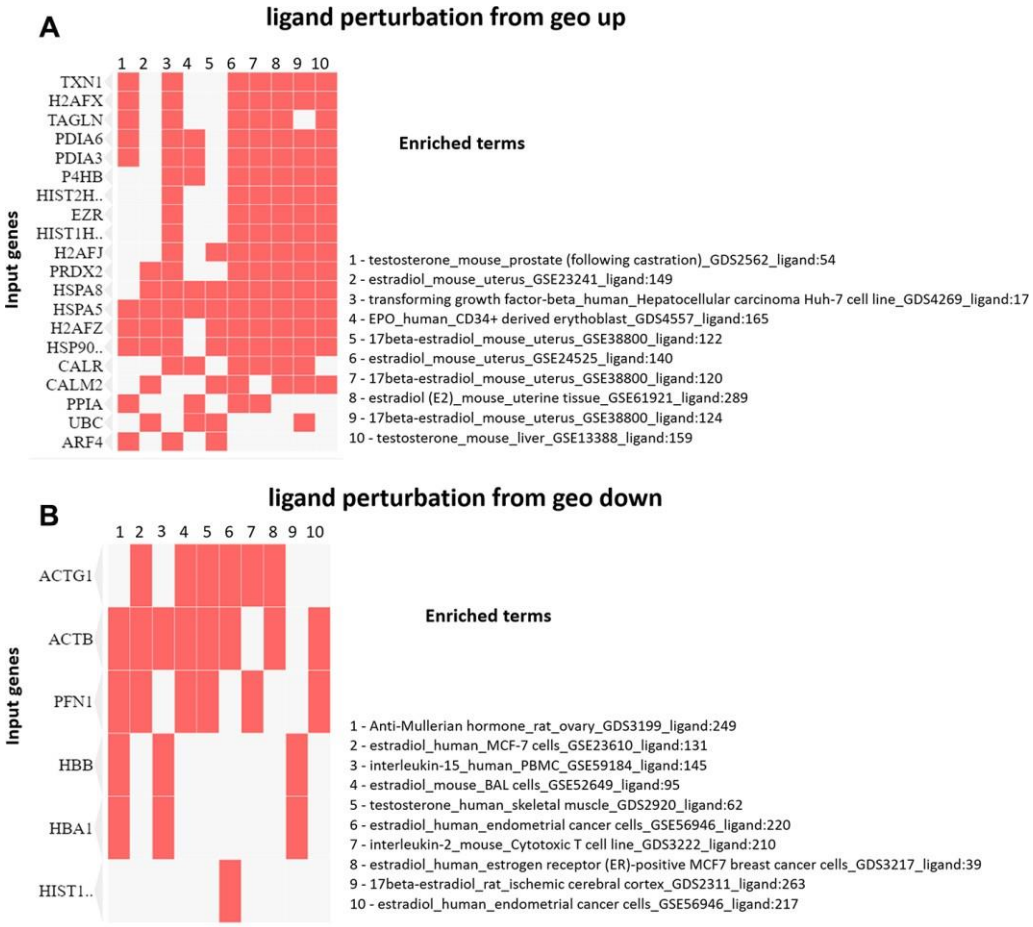

Figure 6. (A) Clustergram generated by Enrichr using upregulated proteins on PND 21. The red cells in the matrix indicate the genes associated with each term. It was demonstrated the top 10 enriched terms with p-value <0.05. (**B**) Clustergram generated by Enrichr using downregulated. proteins on PND 21. The red cells in the matrix indicate the genes associated with each term. It was demonstrated the enriched terms with p-value <0.05 (top 10).

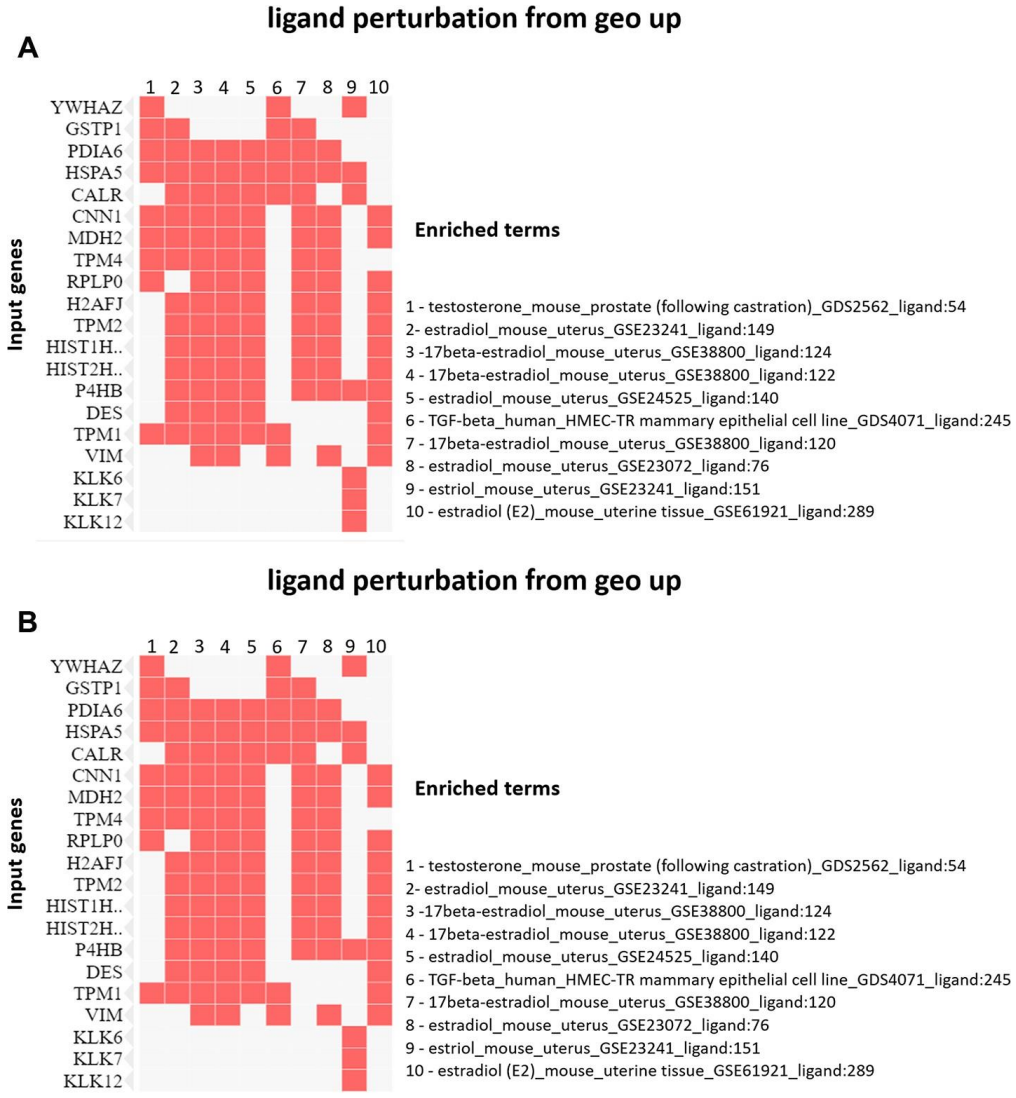

Figure 7. (**A**) Clustergram generated by Enrichr using upregulated proteins on PND 540. The red cells in the matrix indicate the genes associated with each term. It was demonstrated the enriched terms with p-value <0.05 (top 10). (**B**) Clustergram generated by Enrichr using downregulated proteins on PND 540. The red cells in the matrix indicate the genes associated with each term. It was demonstrated the enriched terms with p-value <0.05 (top 10).

Protein-Protein Interaction network

Protein-Protein interaction (PPI) network analysis demonstrated several clusters for up and downregulated proteins on PND 21 and 540 (Supplementary Figures 1– 4). Based on these results, we identified five principal clusters commonly deregulated in both ages: (1) RAB (Ras-related protein) 1, RAB10, RAB15, RAB1A, RAB35, RAB8A, AND RAB8B; (2) H2AFJ (Histone H2A.J), HIST1H2AA, HIST1H2AH, HIST1H2AK, HIST1H2AN, HIST2H2AC, and HIST3H2A; (3) GSTM2 (glutathione S-transferase Mu 2), GSTM4 and GSTM7; (4) CALR (calreticulin), HSPA5 (heat shock protein family A member 5), P4HB (protein disulfide isomerase-4), PDIA6 (Protein Disulfide Isomerase Family A Member 6); (5) PRDX5 (peroxiredoxin-5) and TXN1 (thioredoxin 1). The cluster identified in commonly

downregulated proteins on PND 21 and 540 was composed of HBA1 (hemoglobin Subunit Alpha 1), HBA-A2, HBB, and HBE1 (Figure 8).

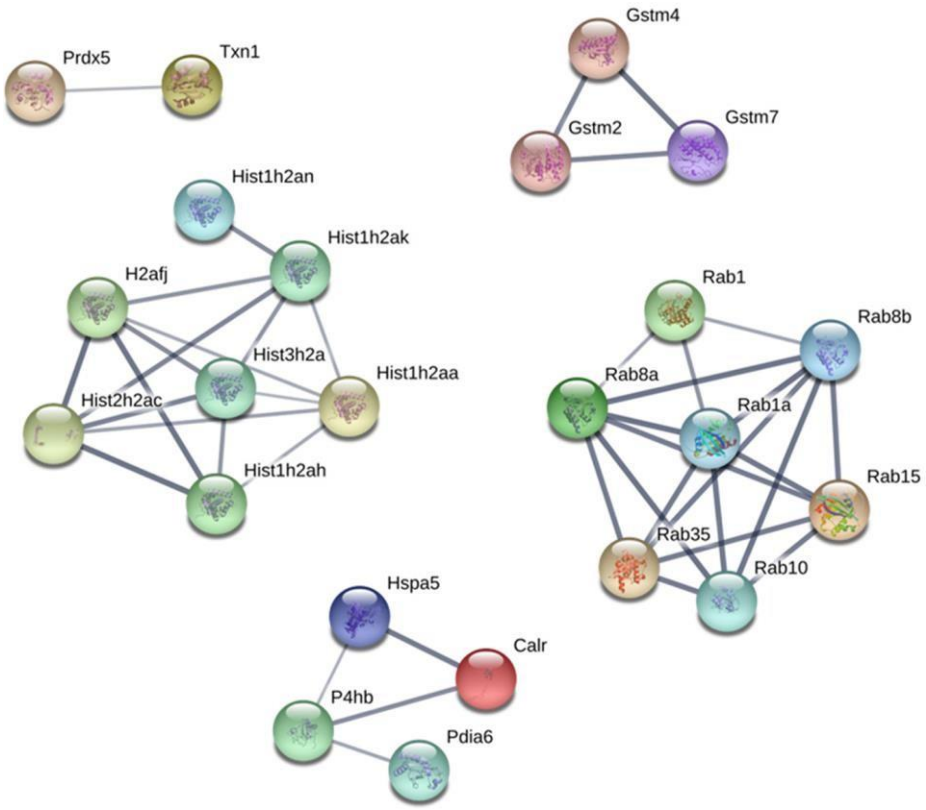

Figure 8. Protein-protein interaction network between commonly upregulated proteins on both PND 21 and 540. Interactions of the identified proteins were mapped by searching the STRING database version 9.0 with a confidence cut-off of 0.7. In the resulting protein association network, proteins are presented as nodes that are connected by lines whose thickness represents the confidence level (0.7-0.9).

In silico analysis confirmed the relationship between differentially expressed proteins (DEP) and PCa in both rodent model and human samples

To give further insights into the role of maternal malnutrition on prostate carcinogenesis, we compared our set of DEP with data from a transgenic PCa mouse model and data from The Cancer Genomic Atlas (TCGA) patients with PCa taken from Gene Expression Profiling Interactive Analysis (GEPIA). In the PB- Cre/PtenloxP/loxP, we identified a set of DEP commonly expressed in our samples, mainly on PND 540 and in the prostatic tumors in all prostatic lobes. Interestingly, the percentage of commonly deregulated targets between our samples and those from the PB-Cre/Ptenl oxP/loxP model increased with aging (from PND 21 to 540) and with the aggressiveness of prostatic lesions (PND 21: PIN 3.6%; medium 5.0%; advanced 6.8% and PND 540: PIN 13.1%; medium 15.9%; advanced 21.3% (Figure 9A). Similar results were obtained when our set of DEP was compared with data from patients with PCa (from 5.9% on PND 21 to 12.0% on PND 540) (Figure 9B). We also identified six proteins (CALR, HIST2H2AC, HSPA5, P4HB, and PDIA6) in the HPA database that showed increased immunostaining in PCa tumor tissue, while

low or not detected in normal prostate tissue (Figure 9C). These results highlight the involvement of maternal malnutrition in the deregulation of proteins involved in prostate tumors.

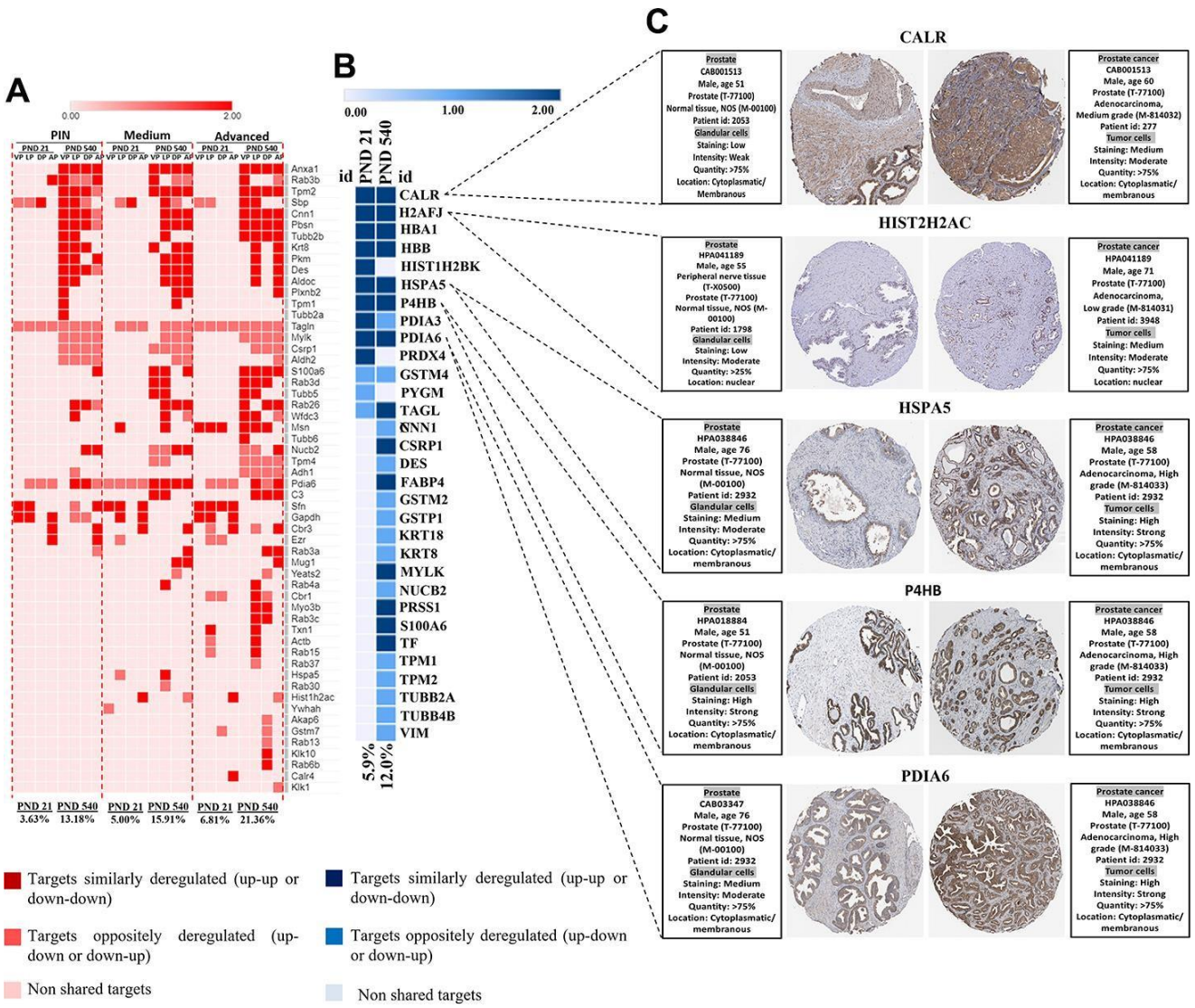

Figure 9. (A) Heatmap showing the commonly differentially targets from our set of DEP and RNA-seq data from ventral, dorsal, lateral, and anterior prostate lobes in the mice model of PCa (PB-Cre/PtenloxP/loxP). The percentage of commonly deregulated targets increases as the prostatic disorders worsen (PIN to Medium to Advanced PCa). (**B**) The commonly deregulated targets between our DEP and those extracted from RNA-seq data by GEPIA. The percentage of commonly deregulated targets increases in the prostate of older offspring. (**C**) Immunostaining of normal and prostate tumor samples for five commonly upregulated targets in our samples and GEPIA database (http://gepia.cancerpku.cn/) using immunohistochemical data available at the Human Protein Atlas database (https://proteinatlas.org/). PND: Postnatal day; VP: Ventral prostate; LP: Lateral prostate; DP: Dorsal prostate; AP: Anterior prostate PIN: Prostate intraepithelial neoplasia; PCa: Prostate cancer.

Experimental validation of CALR as upregulated protein in offspring exposed to maternal malnutrition

Based on the proteomic data (Supplementary File 1) and *in silico* analysis, we employed immunohistochemical and RT-qPCR analyses to validate the CALR as an upregulated target in the offspring VP born to dams fed with LPD in both PND 21 and 540. Immunostaining for CALR was more evident in the GLLP group on both PND 21 (Figure 10C and 10D) and PND 540 (Figure 10G, 10H) compared to the CTR group (Figure 10A, 10B, 10E, 10F) mainly in areas of carcinoma in situ. RT-qPCR confirmed the upregulation of CALR gene expression in the GLLP group at both ages (Figure 10I, 10J).

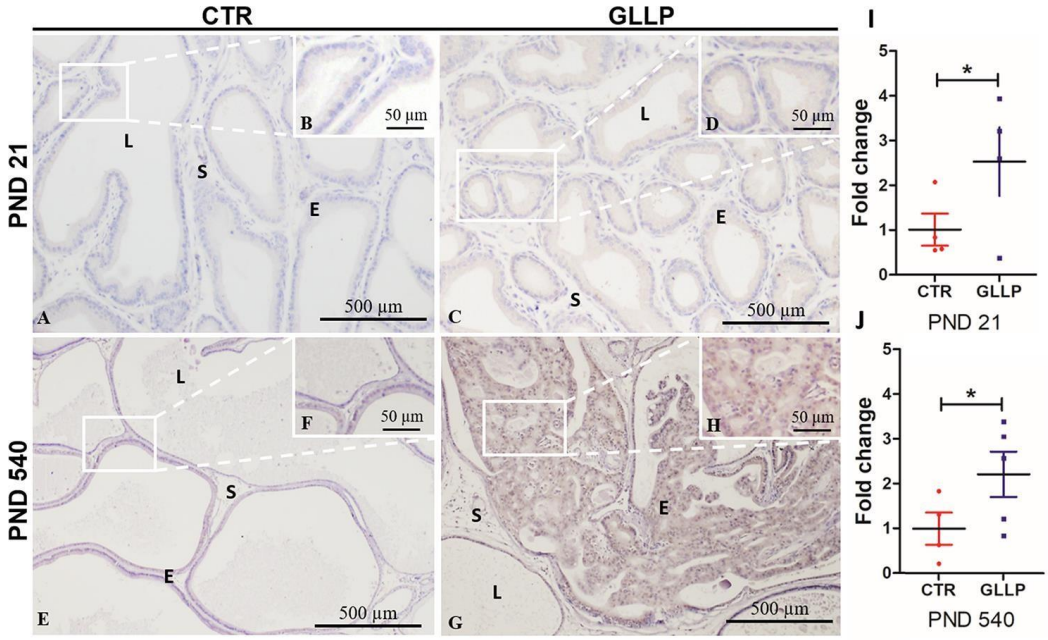

Figure 10. Representative immunohistochemistry reaction for Calreticulin (CALR) in the VP lobes from the CTR and GLLP groups on PND 21 (**A**–**D**) and 540 (**E**–**H**). RT-qPCR reaction for CALR in the VP lobes from CTR and GLLP groups on PND 21 (**I**) and 540 (**J**). CTR = control; GLLP = gestational and lactational low protein; PND = postnatal day. Data are expressed as fold change {plus minus} SD. Asterisks (*) means the statistical difference between experimental groups with $p < .05$. Scale bar: 500 µm, and detail 50 µm.

DISCUSSION

Although maternal exposure to adverse conditions has been identified as an essential window for the development of non-communicable diseases in the progeny, there is a growing body of evidence highlighting malnutrition during early life as a key environmental risk factor for the developmental origin of some types of diseases such as some types of cancer, including breast and PCa in offspring [12–16, 18, 28– 30]. However, little data is supporting the molecular pathways associated with early life carcinogenesis and understanding this aspect may be crucial to identifying and perhaps modulating molecular pathways

involved in the developmental origin of diseases, especially in those more vulnerable populations, who have limited access to more expensive food components, such as proteins [31].

Consistent with our previous results [16, 32], the set of deregulated proteins identified in a mass spectrometry analysis was associated with the molecular mechanism classically recognized as a potent regulator of development, maintenance of tissue homeostasis, and disease. FGFs and EGFs act to regulate glandular morphogenesis, cell proliferation, and differentiation and have secretory functions not only during development but also during neoplastic transformation and tumor progression [33–38]. The Hippo signaling pathway also plays a crucial role in the control of organ size, branching morphogenesis, and tissue homeostasis by regulating cellular mechanisms such as cellular polarity, cell-cell contact, and cytoskeleton organization [39, 40]. The enrichment of molecular pathways related to the endoplasmic reticulum function, such as the metabolism of xenobiotic, chemical carcinogenesis, endocytosis, in addition to cell adhesion, longevity, and apoptosis also highlights the involvement of these cellular and molecular mechanisms in prostatic carcinogenesis [41–45]. The maintenance of these processes is crucial for glandular homeostasis. As a consequence, the breakdown of these molecular mechanisms may also interfere with cell-cell adhesion and metabolism, the maintenance of barriers between the blood and the epithelial and stromal compartments, in addition to interfering within the dynamics of cellular differentiation, proliferation, and migration, all mechanisms involved in the initial stages of carcinogenesis [46–49]. Our data also confirmed the involvement of the estrogen signaling pathway in maternal malnutrition inducing early life prostate carcinogenesis in rat offspring. Previous experimental evidence has demonstrated that early life exposure to exogenous estrogenic compounds, such as BPA and Phthalates, may epigenetically reprogram prostate developmental biology, lead to prostate carcinogenesis with aging [16, 50].

Protein-protein interactions play a crucial role in the control of cellular functions, signal transduction, and metabolism; as such, understanding these interactions may help us to identify molecular mechanisms involved in maternal malnutrition-induced prostatic disorders. The RAB family of proteins, identified by a PPI network analysis, belongs to the RAS (rat sarcoma) superfamily of small GTPase. RAB comprises a family of 66 members (the number of RAB-GTPases is conserved from yeast to humans) [51, 52], which function as molecular regulators essential for the localization and function of the membrane and secretory proteins such as hormones, growth factors, and their membrane receptors. As such, RAB activates several signaling pathways, including the MAPK pathway involved in cell growth and proliferation [53] and the PI3K/AKT/mTOR pathway that stimulates protein synthesis, cell growth, and inhibits apoptosis [54]. Altered expression and the activity of RAB members have been implicated in the development of several disorders, ranging from neurological disorders to diabetes [55]. Aberrant expression of RAB proteins has also been described in multiple cancers, such as lung, brain, and breast. RAB 35, which appeared to be deregulated in our study, can act as an oncogene [56]. The GTPase-deficient RAB35 mutant (RAB35Q67L) activates the PI3K signaling pathway independently of growth factor stimulation and suppresses apoptosis in human embryonic kidney HEK293E cells [57]. The cluster formed CALR, HSPA5, P4HB and, PDIA6 is a potential indicator of maternal malnutrition on the endoplasmic reticulum dysfunction in the prostate of offspring since they act as the fundamental molecular machinery for correct protein folding and Ca^{2+} homeostasis. In a mouse model of caloric restriction (CR), Schafer et al. [58] compared the influence of CR on the hippocampus at younger-adult and older-adult time points and

identified the upregulation of HSPA1B, HSPA5, PDIA4, PDIA6, and CALR. Other authors have also associated the deregulation of these proteins in several types of cancer, including breast carcinoma, hepatoma cells, non-small cell lung cancer, and glioma [59–63]. Although epigenetic modifications of histones, such as histone lysine methylation and demethylation, histone lysine acetylation and deacetylation have been implicated in the modulation of gene expression in the physiological and pathological conditions [64, 65], deregulation of histone expression itself has also been described in several types of malignancies. Xie et al. [66] demonstrated upregulation of hub genes formed by HIST1H1B, HIST1H2AJ, HIST1H2AM, HIST1H2BI, HIST1H2BO, HIST1H3B, HIST1H3F, HIST1H3H, HIST1H4C, and HIST1H4D in breast cancer, indicating that higher expression of these histones was associated with poor overall survival, relapse-free survival, and distant metastasis-free survival. Interestingly, we also observed an increased expression of HIST1 gene members (HIST1H2AA, HIST1H2AH, HIST1H2AK, HIST1H2AN) in the VP of both young and older rats exposed to maternal LPD. This result highlights the potential involvement of the upregulation of histone proteins on prostatic disorders.

Another cluster is formed by enzymes acting in response to oxidative stress as GST, PRDX, and TXN1. The superfamily of GSTs acts in several mechanisms of cellular detoxification, resistance to anticancer drugs, pollutants, and chemicals [67], and the overexpression of GSTs is also associated with the presence of an inflammatory process [68]. It has been demonstrated that low expression of GSTs increases reactive oxygen species in spermatozoids, leading to a degradation of the plasma membrane and a loss of sperm viability [69– 71]. In the prostate gland, GSTs are mainly expressed in the basal cells [72], and their overexpression is associated with epithelial disorders [73], DNA oxidation, and methylation [74, 75]. PRDX-5 is known to act as a redox sensor in the cytosol and several cellular compartments, and silencing it makes the cell more susceptible to DNA damage and apoptosis [76]. In gastric cancer, overexpression of PRDX5 alters the epithelial to mesenchymal transition (EMT) mechanism, with a poor prognosis for patients [77] being correlated. TXN recycles oxidized PRDXs, and this function is essential to balance intracellular oxidative stress [78, 79]. The increased expression of TXN in prostate tissue has been positively correlated with the progression of Gleason score in patients with PCa [80], indicating that transformed cells express higher levels of Trx 1 compared with normal cells. On the other hand, the treatment of prostate cancer cells with natural bioactive compounds reduces TXN expression, collaborating with the apoptosis of these cells [81]. Thus, the high expression of TXN in the prostate of maternal LPD offspring could be related to the development of carcinoma in situ, as observed in older rats.

Considering that groups of interacting proteins are deregulated in both younger and older undernourished offspring, it is possible that histones and RAS-GTPase families and proteins related to oxidative stress, besides those involved with endoplasmic reticulum metabolism and function, may participate in the long-term effect of maternal malnutrition on the prostate of offspring. These results become more relevant with the identification of several of these proteins in patients and mice model of PCa by *in silico* analysis.

CONCLUSIONS

In the present study, we show that maternal exposure to low protein diet deregulated molecular pathways involved in prostate development early in life, which may act as risk factors for prostate

carcinogenesis with aging. Estrogenic signaling pathways, endoplasmic reticulum functions related to detoxification, energy metabolism, and molecular sensors of protein folding and Ca^{2+} homeostasis, besides histone, and RAS-GTPase family of proteins appear to be involved in this process. Knowledge of these factors may raise discussions regarding the role of maternal dietary intervention as a favorable public policy for the lifelong prevention of chronic diseases.

MATERIALS AND METHODS

Animals and experimental design

The detailed experimental design is described by Santos et al. [16]. Briefly, after the determination of pregnancy on gestational day 1 (GD1), pregnant rats were distributed into two experimental groups (n=6/group): Control (CTR): dams fed a normal protein diet (17% protein) and gestational and lactational low protein (GLLP): dams fed a low protein diet (LPD) during gestational and lactational periods. Normal and LPD diets were provided by PragSoluções (PragSoluções, SP, Brazil). All diets were isocaloric and normosodic (Supplementary Table 1). The male offspring were euthanized on a postnatal day (PND) 21 (weaning) (n=12/group) and PND 540 (n=12/group). The offspring, which were euthanized on PND 540, had free access to a normal protein diet after weaning until the end of the experiment. The animals were euthanized by an overdose of anesthesia (ketamine/xylazine) followed by decapitation, weighing, and the blood and ventral prostate (VP) were collected and processed by a different analysis as described below. The body weight, and VP weight, and hormonal levels were analyzed using a Student t-test, and statistical differences were considered when $p < 0.05$. The animal procedures were approved by the Biosciences Institute/UNESP Ethics Committee for Animal Experimentation (Protocol #573) following the ethical animal research principles and the Brazilian legislation established by the Brazilian Council of Control in Animal Experimentation.

Hormone analysis

Blood samples from offspring (n=12/group) were centrifuged (2400 g for 20 minutes), and sera were used to determine the concentrations of estrogen (17β- estradiol, Monobind®, 4925-300 CA, USA sensitivity: 6.5 pg/mL) and testosterone (17β-hydroxy-4- androstene-3-one, Monobind®, 3725-300A, CA. sensitivity: 0.038 ng/mL). The hormonal qualifications were determined in 96-well plates using the ELISA plate reader (Epoch™, Biotek Instruments, VT, USA) following the manufacturers' protocol.

Selection of prostate samples for mass spectrometry analysis

In a previous study, Santos et al. [16] demonstrated that maternal exposure to LPD induced a delay in prostatic growth on PND 21, which was associated with prostate carcinogenesis in older rats on PND 540. Slides of the left VP lobes (n=3/group) were stained with hematoxylin-eosin (HE) and analyzed using a Leica DMLB 80 microscope To exemplify the histological characteristics of the VP lobes from the CTR and GLLP groups on PND 21 and 540. Based on these results, the contralateral right VP lobes (n=3/group) from each group were submitted to mass spectrometry.

Immunohistochemistry

Histological sections of 5 μm (n = 6 per group) were processed as described by Santos et al. [16]. After the initial steps, the slides were boiled for 30 min in 10 mM sodium citrate solution (pH 6.0) for antigen retrieval. Prostatic sections were blocked in 5% nonfat milk diluted in phosphate-buffered saline (PBS) and incubated with anti-Calreticulin antibody (ab2908) specific primary antibody overnight at 4°C. Slides were washed in PBS and incubated for one hour at room temperature in horseradish peroxidase (HRP)- conjugated secondary antibody. The slides were washed, and the reaction was developed using 3,3′-Diaminobenzidine (DAB, Sigma) and counterstained with hematoxylin for 30 seconds. The reactions were analyzed using a Leica DMLB 80 microscope.

RT-qPCR

Prostate samples (n= 6 per group) from all experimental groups on PND 21 and PND 540 were used to total RNA extraction using TRIzol® Reagent (ThermoFisher aScientific) according to the manufacturer's recommendations. RNA integrity was evaluated by capillary electrophoresis using a 2100 Bioanalyzer (Agilent). Only samples with an RNA integrity number (RIN) ≥ 7.0 were used. The extracted RNA was treated with DNase I (Amplification Grade; ThermoFisher Scientific). The synthesis of cDNA was performed using a High-Capacity cDNA Archive Kit (ThermoFisher Scientific) according to the manufacturer's guidelines. Expression levels of CALR mRNA were measured by RT-qPCR using the QuantStudio™ 12K Flex Real-Time PCR System (Thermo Fisher Scientific). All qPCRs performed were compliant with the Minimum Information for Publication of Quantitative Real-Time PCR experiments (MIQE) guidelines [82]. The cDNA samples were amplified using SYBR® Green Master Mix (ThermoFisher Scientific), and specific primers were synthesized by Invitrogen to the CALR gene, forward: GCCAGACACTGGTGGTACAGTTC reverse: CGCCC CCACAGTCGATATT. Relative quantification of expression was performed by the $2^{-\Delta\Delta Ct}$ method [83] using DataAssistTM v3.01 software (Thermo Fisher Scientific). According to the expression stability among all samples, the reference gene GUSB (β-glucuronidase) and GAPDH (glyceraldehyde 3-phosphate dehydrogenase) were used to normalize mRNA expression.

Mass spectrometry

The mass spectrometry protocol was based on a previous study by Gabriel Kuniyoshi et al. [84], Dionizio et al. [85], and Da Silva-Gomes et al. [86], with modifications. Briefly, protein extraction was carried out by homogenizing three VPs lobes from each experimental group on PND 21 and 540 in extraction buffer containing 0.01 M Tris-HCl, 0.005 M phenylmethylsulfonyl fluoride, 1% protease inhibitor, 0.065 M dithiothreitol, 8 M urea (in a proportion of 30mg tissue/100 μg buffer). The homogenate was vortexed for 2-3 min and centrifuged for 15 min at 9,690 g and 4°C. The supernatant was recovered, and the total protein was quantified by Bradford assay using the BSA standard [87]. Samples were grouped to constitute three pools of 50 μg proteins each in a total of 50 μL (1 μg/μL). Next, the samples were incubated for 60 min at 37°C with 10 μL of 50 mM ammonium bicarbonate and 25 μl of 0.2% surfactant solution, followed by incubation with 2.5 μL of 0.1 M dithiothreitol for 40 min at 37°C. Carbamidomethylation was performed with 2.5 μL of 0.3 M iodoacetamide, incubated for 30 min at

room temperature, and protected from light. Then, samples were subjected to proteolytic digestion overnight at 37°C using 0.05 µg/µL trypsin diluted in 0.05 M Ammonium bicarbonate, followed by incubation with 10 µL trifluoroacetic acid 5% for 90 min at 37°C. The samples were centrifuged at 14,000 RPM 4ºC for 30 minutes. After this step, the samples were desalted using Sep-Pak Vac C18 (Waters Manchester, UK) columns, reduced in a concentrator, and maintained at - 20°C until the time of analysis by mass spectrometry.

The analysis of the tryptic peptide was performed using the nanoACQUITY UPLC system (Waters, Manchester, UK) coupled to a Xevo Q-TOF G2 mass spectrometer (Waters, Manchester, UK) equipped with nanoACQUITY HSS T3, analytical reverse-phase column (75µmX150 mm, 1.8µm particle size, Waters) previously equilibrated with 7% of mobile phase B (100% ACN + 0.1% formic acid). The peptides were separated by a linear gradient of 7-85% mobile phase B for 70 min at a flow rate of 0,35 µL/min, and the column temperature was maintained at 45°C. The MS was operated in positive ion mode, with a data acquisition time of 75 min. The data obtained were processed using the software Protein Lynx Global Server (PLGS) version 3.03 (Waters Co., Manchester, UK). Protein identification was obtained using an ion count algorithm incorporated into the software. The data obtained were searched in the database of the species *Rattus norvegicus* downloaded from the UniProt catalog (Universal Protein Resource) in December 2017 (https://www.uniprot.org/). Differentially expressed proteins (DEP) between experimental groups were obtained using PLGS software, considering $p < 0.05$ for downregulated proteins and $p > 0.95$ for upregulated proteins.

Functional annotation analysis

KOBAS 3.0 (http://kobas.cbi.pku.edu.cn/) was used to determine the enrichment pathways related to our DEP in the KEGG (https://www.genome.jp/kegg/) and PANTHER (http://pantherdb.org) databases. The cut-off criterion used was an adjusted p-value < 0.05. Also, we used the Ligand Perturbation database from the Enrichr tool (http://amp.pharm.mssm.edu/Enrichr) to compare the set of DEP from those extracted from GEO comparing human or mouse cells before and after treatment with endogenous ligands. We used the top 10 most enriched terms with a p-value < 0.05 [88]. The STRING tool (http://string-db.org/) was used to construct the protein-protein interaction (PPI) network associated with our DEP by searching neighbor interactors with our imputed proteins. To avoid false positive interactions, we selected a high confidence score (0.7), associated with experiments and a database as two stringent evidence channels [89].

Relevance of deregulated proteins in human and mouse model of PCa: in silico validation

To give further insights into the relationship between maternal malnutrition and PCa, we compared our set of DEP with RNA-seq data from a transgenic mice model for PCa: PB-Cre/Pten$^{loxP/loxP}$. In this study, Jurmeister et al. [90] described data from the RNA-seq of four prostate lobes (ventral, anterior, dorsal, and lateral) at different stages of tumorigenesis: low-grade prostate intraepithelial neoplasia (PIN), medium-stage tumors (Medium) and advanced-stage tumors (Advanced). The dataset was downloaded from the NCBI Gene Expression Omnibus (https://www.ncbi.nlm.nih. gov/geo/), accession number GSE94574. We considered differentially expressed genes: < -1.3 Log2FC > 1.3, adjusted p-value < 0.05. We also identified differentially expressed genes between normal from Genotype-Tissue Expression (GTEx) with 221

patients/samples and PCa human samples extracted from RNA-seq data using Prostate Adenocarcinoma (TCGA, PanCancer Atlas) with 488 patients/samples data analyzed using the GEPIA database (Gene Expression Profiling Interactive Analysis) (http:// gepia.cancer-pku.cn/) [91]. We considered differently expressed genes with < -1 Log2FC > 1 and q-value < 0.05. This set of genes was compared with our DEP to identify possible molecular mechanisms shared by our samples and those from human PCa. Additionally, the commonly upregulated proteins identified in our sample and RNA-seq of prostatic tumor samples identified by GEPIA were submitted to The Human Protein Atlas (HPA) (https://www.proteinatlas.org/) database to demonstrate the distribution and localization of these proteins in normal and tumor samples by immunohistochemistry.

Data representation and analyses

Bar plots were constructed using GraphPad Prism (GraphPad Software). We used the webserver http://bioinformatics.psb.ugent.be/webtools/Venn/ to plot the Venn diagrams. Heat maps were created using the web tool Morpheus [92] (https://software. broadinstitute.org/morpheus), and circus plots were generated in environment R with package 'circlize' [93].

Abbreviations

CALR: Calreticulin;

CR: Caloric restriction;

CTR: Control group;

DAB: 3,3′-Diaminobenzidine;

DEP: Differentially expressed proteins;

DOHaD: developmental origins of health and disease;

EGF: Epidermal growth factor;

EMT: Epithelial to mesenchymal transition;

FGF: Fibroblast growth factor;

FOAD: Fetal Origin of Adult Diseases;

GAPDH: Glyceraldehyde 3-phosphate dehydrogenase;

GD: Gestational day;

GEPIA: Gene Expression Profiling Interactive Analysis;

GLLP: Gestational and lactational low protein group;

GLOBOCAN: Global Cancer Statistics;

GSTM2: Glutathione S-transferase Mu 2;

GTEx: Genotype-Tissue Expression; GUSB: β- glucuronidase;

H2AFJ: Histone H2A.J;

HBA1: Hemoglobin Subunit Alpha 1;

HE: Hematoxylin–eosin; HPA: Human Protein Atlas;

HSPA5: Heat shock protein family A member 5;

LPD: Low Protein Diet; MAPK:

Mitogen-activated protein kinase;

MM: Maternal malnutrition;

P4HB: Protein disulfide isomerase-4; PBS: Phosphate-buffered saline;

PCa: Prostate cancer;

PDIA6: Protein Disulfide Isomerase Family A Member 6;

PI3K: Phosphatidylinositol 3' - kinase; PIN: Intraepithelial neoplasia;

PND: Post-natal day; PPI:

Protein-Protein interaction;

PRDX5: Peroxiredoxin-5;

RAB: Ras-related protein;

RAP1: Ras- proximate-1; RAS: Rat sarcoma;

RIN: RNA integrity number;

TCGA: The Cancer Genomic Atlas;

TXN1: Thioredoxin 1;

UniProt: Universal Protein Resource;

VP: Ventral prostate.

AUTHOR CONTRIBUTIONS

Conceptualization, S.A.A.S., and L.A.J.; methodology, S.A.A.S., A.C.L.C., F.B.C., KT.C., M.N.F, J.C.S.V.,P.G.P., L.M.F.P., M.B, L.A.J.; formal analysis, S.A.A.S., L.A.J.; investigation, S.A.A.S., A.C.C.,F.B.C., L.A.J.; resources, S.A.A.S., A.C.L.C., L.A.J; data curation, S.A.A.S., S.L.F., L.A.J.; writing— original draft preparation, S.A.A.S., A.C.L.C., F.B.C., L.A.J.; writing—review and editing, all authors; supervision, L.A.J; project administration, L.A.J.; funding acquisition, L.A.J.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

1. Chatterji S, Byles J, Cutler D, Seeman T, Verdes E. Health, functioning, and disability in older adults— present status and future implications. Lancet. 2015; 385:563–75. https://doi.org/10.1016/S0140-6736(14)61462-8 PMID:25468158

2. Newman CB, Preiss D, Tobert JA, Jacobson TA, Page RL 2n, Goldstein LB, Chin C, Tannock LR, Miller M,Raghuveer G, Duell PB, Brinton EA, Pollak A, et al, and American Heart Association Clinical Lipidology, Lipoprotein, Metabolism and Thrombosis Committee, a Joint Committee of the Council on Atherosclerosis, Thrombosis and Vascular Biology and Council on Lifestyle and Cardiometabolic Health; Council on Cardiovascular Disease in the Young; Council on Clinical Cardiology; and Stroke Council. Statin safety and associated adverse events: a scientific statement from the American heart association. Arterioscler Thromb Vasc Biol. 2019; 39:e38–81. https://doi.org/10.1161/ATV.0000000000000073 PMID:30580575

3. Barker DJ, Osmond C, Golding J, Kuh D, Wadsworth ME. Growth in utero, blood pressure in childhood and adult life, and mortality from cardiovascular disease. BMJ. 1989; 298:564–67. https://doi.org/10.1136/bmj.298.6673.564 PMID:2495113

4. Dickinson FM, Pyone T, van den Broek N. Experiences from the field: maternal, reproductive and child health data collection in humanitarian and emergency situations. Int Health. 2016; 8:83–88. https://doi.org/10.1093/inthealth/ihv045 PMID:26188190

5. McKerracher L, Moffat T, Barker M, McConnell M, Atkinson SA, Murray-Davis B, McDonald SD, Sloboda DM. Knowledge about the developmental origins of health and disease is independently associated with variation in diet quality during pregnancy. Matern Child Nutr. 2020; 16:e12891. https://doi.org/10.1111/mcn.12891 PMID:31833216

6. Barker DJ, Bull AR, Osmond C, Simmonds SJ. Fetal and placental size and risk of hypertension in adult life. BMJ. 1990; 301:259–62. https://doi.org/10.1136/bmj.301.6746.259 PMID:2390618

7. Wadhwa PD, Buss C, Entringer S, Swanson JM. Developmental origins of health and disease: brief history of the approach and current focus on epigenetic mechanisms. Semin Reprod Med. 2009; 27:358–68. https://doi.org/10.1055/s-0029-1237424 PMID:19711246

8. Schulz LC. The dutch hunger winter and the developmental origins of health and disease. Proc Natl Acad Sci USA. 2010; 107:16757–58. https://doi.org/10.1073/pnas.1012911107 PMID:20855592

9. Lumey LH, Van Poppel FW. The dutch famine of 1944- 45: mortality and morbidity in past and present generations. Soc Hist Med. 1994; 7:229–46. https://doi.org/10.1093/shm/7.2.229 PMID:11639327

10. Hoek HW, Susser E, Buck KA, Lumey LH, Lin SP, Gorman JM. Schizoid personality disorder after prenatal exposure to famine. Am J Psychiatry. 1996; 153:1637–39. https://doi.org/10.1176/ajp.153.12.1637 PMID:8942466

11. Kahn HS, Stein AD, Lumey LH. Prenatal environmental exposures that may influence β-cell function or insulin sensitivity in middle age. J Dev Orig Health Dis. 2010; 1:300–09. https://doi.org/10.1017/S2040174410000474 PMID:25141933

12. Walker CL, Ho SM. Developmental reprogramming of cancer susceptibility. Nat Rev Cancer. 2012; 12:479–86. https://doi.org/10.1038/nrc3220 PMID:22695395

13. Keinan-Boker L, Vin-Raviv N, Liphshitz I, Linn S, Barchana M. Cancer incidence in Israeli jewish survivors of world war II. J Natl Cancer Inst. 2009; 101:1489–500. https://doi.org/10.1093/jnci/djp327 PMID:19861305

14. Barker DJ, Osmond C, Thornburg KL, Kajantie E, Eriksson JG. A possible link between the pubertal growth of girls and prostate cancer in their sons. Am J Hum Biol. 2012; 24:406–10. https://doi.org/10.1002/ajhb.22222 PMID:22287160

15. Varuzza MB, Zapaterini JR, Colombelli KT, Barquilha CN, Justulin LA Jr, Muñoz-de-Toro M, Kass L, Barbisan LF. Impact of gestational low protein diet and postnatal bisphenol a exposure on chemically induced mammary carcinogenesis in female offspring rats. Environ Toxicol. 2019; 34:1263–72. https://doi.org/10.1002/tox.22827 PMID:31287222

16. Santos SA, Camargo AC, Constantino FB, Colombelli KT, Mani F, Rinaldi JC, Franco S, Portela LM, Duran BO, Scarano WR, Hinton BT, Felisbino SL, Justulin LA. Maternal low-protein diet impairs prostate growth in young rat offspring and induces prostate carcinogenesis with aging. J Gerontol A Biol Sci Med Sci. 2019; 74:751–59. https://doi.org/10.1093/gerona/gly118 PMID:29762647

17. Scarano WR, Bedrat A, Alonso-Costa LG, Aquino AM, Fantinatti B, Justulin LA, Barbisan LF, Freire PP, Flaws JA, Bernardo L. Exposure to an environmentally relevant phthalate mixture during prostate development induces microRNA upregulation and transcriptome modulation in rats. Toxicol Sci. 2019; 171:84–97. https://doi.org/10.1093/toxsci/kfz141 PMID:31199487

18. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018; 68:394–424. https://doi.org/10.3322/caac.21492 PMID:30207593

19. Gardner WA. Hypothesis: the prenatal origins of prostate cancer. Hum Pathol. 1995; 26:1291–92. https://doi.org/10.1016/0046-8177(95)90291-0 PMID:8522299

20. Powell IJ, Meyskens FL Jr. African American men and hereditary/familial prostate cancer: intermediate-risk populations for chemoprevention trials. Urology. 2001; 57:178–81. https://doi.org/10.1016/s0090-4295(00)00968-7 PMID:11295621

21. Henderson BE, Bernstein L, Ross RK, Depue RH, Judd HL. The early in utero oestrogen and testosterone environment of blacks and whites: potential effects on male offspring. Br J Cancer. 1988; 57:216–18. https://doi.org/10.1038/bjc.1988.46 PMID:3358915

22. Elias SG, Peeters PH, Grobbee DE, van Noord PA. Breast cancer risk after caloric restriction during the 1944-1945 dutch famine. J Natl Cancer Inst. 2004; 96:539–46. https://doi.org/10.1093/jnci/djh087 PMID:15069116

23. Dirx MJ, van den Brandt PA, Goldbohm RA, Lumey LH. Energy restriction in childhood and adolescence and risk of prostate cancer: results from the Netherlands cohort study. Am J Epidemiol. 2001; 154:530–37. https://doi.org/10.1093/aje/154.6.530 PMID:11549558

24. Ho SM, Tang WY, Belmonte de Frausto J, Prins GS. Developmental exposure to estradiol and bisphenol a increases susceptibility to prostate carcinogenesis and epigenetically regulates phosphodiesterase type 4 variant 4. Cancer Res. 2006; 66:5624–32. https://doi.org/10.1158/0008-5472.CAN-06-0516 PMID:16740699

25. Prins GS, Hu WY, Xie L, Shi GB, Hu DP, Birch L, Bosland MC. Evaluation of bisphenol a (BPA) exposures on prostate stem cell homeostasis and prostate cancer risk in the NCTR-sprague-dawley rat: an NIEHS/FDA CLARITY-BPA consortium study. Environ Health Perspect. 2018; 126:117001. https://doi.org/10.1289/EHP3953 PMID:30387366

26. Prins GS, Ye SH, Birch L, Zhang X, Cheong A, Lin H, Calderon-Gierszal E, Groen J, Hu WY, Ho SM, van Breemen RB. Prostate cancer risk and DNA methylation signatures in aging rats following developmental BPA exposure:a dose-response analysis. Environ Health Perspect. 2017; 125:077007. https://doi.org/10.1289/EHP1050 PMID:28728135

27. Cheong JN, Wlodek ME, Moritz KM, Cuffe JS. Programming of maternal and offspring disease: impact of growth restriction, fetal sex and transmission across generations. J Physiol. 2016; 594:4727–40. https://doi.org/10.1113/JP271745 PMID:26970222

28. Trichopoulos D, MacMahon B, Brown J. Socioeconomic status, urine estrogens, and breast cancer risk. J Natl Cancer Inst. 1980; 64:753–55. PMID:6928988

29. Justulin LA, Dos Santos SAA, Damasceno DC, Scarano WR, Felisbino SL. Chapter 20: Nutrition and prostate

cancer prevention. Food Chemistry, Function and Analysis. Royal Society of Chemistry; 2020; 21:392–411. https://doi.org/10.1039/9781788016506-00392

30. de Mello Santos T, Cavariani MM, Pereira DN, Schimming BC, Chuffa LG, Domeniconi RF. Maternal protein restriction modulates angiogenesis and AQP9 expression leading to a delay in postnatal epididymal development in rat. Cells. 2019; 8:1094. https://doi.org/10.3390/cells8091094 PMID:31533210

31. Barbeito-Andrés J, Pezzuto P, Higa LM, Dias AA, Vasconcelos JM, Santos TM, Ferreira JC, Ferreira RO, Dutra FF, Rossi AD, Barbosa RV, Amorim CK, De Souza MP, et al. Congenital zika syndrome is associated with maternal protein malnutrition. Sci Adv. 2020; 6:eaaw6284. https://doi.org/10.1126/sciadv.aaw6284 PMID:31950075

32. Colombelli KT, Santos SA, Camargo AC, Constantino FB, Barquilha CN, Rinaldi JC, Felisbino SL, Justulin LA. Impairment of microvascular angiogenesis is associated with delay in prostatic development in rat offspring of maternal protein malnutrition. Gen Comp Endocrinol. 2017; 246:258–69. https://doi.org/10.1016/j.ygcen.2016.12.016 PMID:28041790

33. Kim HG, Kassis J, Souto JC, Turner T, Wells A. EGF receptor signaling in prostate morphogenesis and tumorigenesis. Histol Histopathol. 1999; 14:1175–82. https://doi.org/10.14670/HH-14.1175 PMID:10506934

34. Donjacour AA, Thomson AA, Cunha GR. FGF-10 plays an essential role in the growth of the fetal prostate. Dev Biol. 2003; 261:39–54. https://doi.org/10.1016/s0012-1606(03)00250-1 PMID:12941620

35. Ma Y, Kakudo N, Morimoto N, Lai F, Taketani S, Kusumoto K. Fibroblast growth factor-2 stimulates proliferation of human adipose-derived stem cells via src activation. Stem Cell Res Ther. 2019; 10:350. https://doi.org/10.1186/s13287-019-1462-z PMID:31775870

36. Thomson AA, Cunha GR. Prostatic growth and development are regulated by FGF10. Development. 1999; 126:3693–701.
PMID:10409514

37. Pillai A, Patel S, Ranadive I, Desai I, Balakrishnan S. Fibroblast growth factor-2 signaling modulates matrix reorganization and cell cycle turnover rate in the regenerating tail of hemidactylus flaviviridis. Acta Histochem. 2020; 122:151464. https://doi.org/10.1016/j.acthis.2019.151464 PMID:31780191

38. Ropiquet F, Giri D, Lamb DJ, Ittmann M. FGF7 and FGF2 are increased in benign prostatic hyperplasia and are associated with increased proliferation. J Urol. 1999; 162:595–99. PMID:10411093

39. Meng Z, Moroishi T, Guan KL. Mechanisms of hippo pathway regulation. Genes Dev. 2016; 30:1–17. https://doi.org/10.1101/gad.274027.115 PMID:26728553

40. Hong AW, Meng Z, Guan KL. The hippo pathway in intestinal regeneration and disease. Nat Rev Gastroenterol Hepatol. 2016; 13:324–37. https://doi.org/10.1038/nrgastro.2016.59 PMID:27147489

41. Zacharopoulou N, Tsapara A, Kallergi G, Schmid E, Tsichlis PN, Kampranis SC, Stournaras C. The epigenetic factor KDM2B regulates cell adhesion, small rho GTPases, actin cytoskeleton and migration in prostate cancer cells. Biochim Biophys Acta Mol Cell Res. 2018; 1865:587–97. https://doi.org/10.1016/j.bbamcr.2018.01.009 PMID:29408056

42. Mason MD, Davies G, Jiang WG. Cell adhesion molecules and adhesion abnormalities in prostate cancer. Crit Rev Oncol Hematol. 2002; 41:11–28. https://doi.org/10.1016/s1040-8428(01)00171-8 PMID:11796229

43. Albany C, Hahn NM. Heat shock and other apoptosis- related proteins as therapeutic targets in prostate cancer. Asian J Androl. 2014; 16:359–63. https://doi.org/10.4103/1008-682X.126400 PMID:24713836

44. Giampietri C, Petrungaro S, Conti S, Facchiano A, Filippini A, Ziparo E. Cancer microenvironment and endoplasmic reticulum stress response. Mediators Inflamm. 2015; 2015:417281. https://doi.org/10.1155/2015/417281 PMID:26491226

45. Jaud M, Philippe C, Di Bella D, Tang W, Pyronnet S, Laurell H, Mazzolini L, Rouault-Pierre K, Touriol C. Translational regulations in response to endoplasmic reticulum stress in cancers. Cells. 2020; 9:540. https://doi.org/10.3390/cells9030540 PMID:32111004

46. Knudsen BS, Vasioukhin V. Mechanisms of prostate cancer initiation and progression. Adv Cancer Res. 2010; 109:1–50. https://doi.org/10.1016/B978-0-12-380890-5.00001-6 PMID:21070913

47. Shiao SL, Chu GC, Chung LW. Regulation of prostate cancer progression by the tumor microenvironment. Cancer Lett. 2016; 380:340–48. https://doi.org/10.1016/j.canlet.2015.12.022 PMID:26828013

48. Humphrey PA. Histopathology of prostate cancer. Cold Spring Harb Perspect Med. 2017; 7:a030411. https://doi.org/10.1101/cshperspect.a030411 PMID:28389514

49. Hütz K, Zeiler J, Sachs L, Ormanns S, Spindler V. Loss of desmoglein 2 promotes tumorigenic behavior in pancreatic cancer cells. Mol Carcinog. 2017; 56:1884–95. https://doi.org/10.1002/mc.22644 PMID:28277619

50. Prins GS, Ho SM. Early-life estrogens and prostate cancer in an animal model. J Dev Orig Health Dis. 2010; 1:365–

70. https://doi.org/10.1017/S2040174410000577 PMID:24795802

51. Stenmark H, Olkkonen VM. The rab GTPase family. Genome Biol. 2001; 2:REVIEWS3007. https://doi.org/10.1186/gb-2001-2-5-reviews3007 PMID:11387043

52. Li G, Marlin MC. Rab family of GTPases. Methods Mol Biol. 2015; 1298:1–15. https://doi.org/10.1007/978-1-4939-2569-8_1 PMID:25800828

53. Nan X, Tamgüney TM, Collisson EA, Lin LJ, Pitt C, Galeas J, Lewis S, Gray JW, McCormick F, Chu S. ras-GTP dimers activate the mitogen-activated protein kinase (MAPK) pathway. Proc Natl Acad Sci USA. 2015; 112:7996–8001. https://doi.org/10.1073/pnas.1509123112 PMID:26080442

54. Castellano E, Downward J. RAS interaction with PI3K: more than just another effector pathway. Genes Cancer. 2011; 2:261–74. https://doi.org/10.1177/1947601911408079 PMID:21779497

55. Abd Elmageed ZY, Yang Y, Thomas R, Ranjan M, Mondal D, Moroz K, Fang Z, Rezk BM, Moparty K, Sikka SC, Sartor O, Abdel-Mageed AB. Neoplastic reprogramming of patient-derived adipose stem cells by prostate cancer cell-associated exosomes. Stem Cells. 2014; 32:983–97. https://doi.org/10.1002/stem.1619 PMID:24715691

56. Gopal Krishnan PD, Golden E, Woodward EA, Pavlos NJ, Blancafort P. Rab GTPases: emerging oncogenes and tumor suppressive regulators for the editing of survival pathways in cancer. Cancers (Basel). 2020; 12:259. https://doi.org/10.3390/cancers12020259 PMID:31973201

57. Wheeler DB, Zoncu R, Root DE, Sabatini DM, Sawyers CL. Identification of an oncogenic RAB protein. Science. 2015; 350:211–17. https://doi.org/10.1126/science.aaa4903 PMID:26338797

58. Schafer MJ, Dolgalev I, Alldred MJ, Heguy A, Ginsberg SD. Calorie restriction suppresses age-dependent hippocampal transcriptional signatures. PLoS One. 2015; 10:e0133923. https://doi.org/10.1371/journal.pone.0133923 PMID:26221964

59. Bini L, Magi B, Marzocchi B, Arcuri F, Tripodi S, Cintorino M, Sanchez JC, Frutiger S, Hughes G, Pallini V, Hochstrasser DF, Tosi P. Protein expression profiles in human breast ductal carcinoma and histologically normal tissue. Electrophoresis. 1997; 18:2832–41. https://doi.org/10.1002/elps.1150181519 PMID:9504817

60. Bai Y, Wang G, Fu W, Lu Y, Wei W, Chen W, Wu X, Meng H, Feng Y, Liu Y, Li G, Wang S, Wang K, et al. Circulating essential metals and lung cancer: Risk assessment and potential molecular effects. Environ Int. 2019; 127:685–693. https://doi.org/10.1016/j.envint.2019.04.021 PMID:30991224

61. Yu LR, Zeng R, Shao XX, Wang N, Xu YH, Xia QC. Identification of differentially expressed proteins between human hepatoma and normal liver cell lines by two-dimensional electrophoresis and liquid chromatography-ion trap mass spectrometry. Electrophoresis. 2000; 21:3058–68. https://doi.org/10.1002/1522-2683(20000801)21:14 <3058::AID-ELPS3058>3.0.CO;2-U PMID:11001323

62. Zou H, Wen C, Peng Z, Shao YY, Hu L, Li S, Li C, Zhou HH. P4HB and PDIA3 are associated with tumor progression and therapeutic outcome of diffuse gliomas. Oncol Rep. 2018; 39:501–10. https://doi.org/10.3892/or.2017.6134 PMID:29207176

63. Albakova Z, Armeev GA, Kanevskiy LM, Kovalenko EI, Sapozhnikov AM. HSP70 multi-functionality in cancer. Cells. 2020; 9:587. https://doi.org/10.3390/cells9030587 PMID:32121660

64. Shanmugam MK, Arfuso F, Arumugam S, Chinnathambi A, Jinsong B, Warrier S, Wang LZ,Kumar AP, Ahn KS, Sethi G, Lakshmanan M. Role of novel histone modifications in cancer. Oncotarget. 2017; 9:11414–26. https://doi.org/10.18632/oncotarget.23356 PMID:29541423

65. Audia JE, Campbell RM. Histone modifications and cancer. Cold Spring Harb Perspect Biol. 2016; 8:a019521. https://doi.org/10.1101/cshperspect.a019521 PMID:27037415

66. Xie W, Zhang J, Zhong P, Qin S, Zhang H, Fan X, Yin Y, Liang R, Han Y, Liao Y, Yu X, Long H, Lv Z, et al. Expression and potential prognostic value of histone family gene signature in breast cancer. Exp Ther Med. 2019; 18:4893–903. https://doi.org/10.3892/etm.2019.8131 PMID:31772649

67. Hayes JD, Flanagan JU, Jowsey IR. Glutathione transferases. Annu Rev Pharmacol Toxicol. 2005; 45:51–88. https://doi.org/10.1146/annurev.pharmtox.45.120403.095857 PMID:15822171

68. Bogdani M, Henschel AM, Kansra S, Fuller JM, Geoffrey R, Jia S, Kaldunski ML, Pavletich S, Prosser S, Chen YG, Lernmark A, Hessner MJ. Biobreeding rat islets exhibit reduced antioxidative defense and n-acetyl cysteine treatment delays type 1 diabetes. J Endocrinol. 2013; 216:111–23. https://doi.org/10.1530/JOE-12-0385 PMID:23111281

69. Llavanera M, Mateo-Otero Y, Bonet S, Barranco I, Fernández-Fuertes B, Yeste M. The triple role of glutathione s-transferases in mammalian male fertility. Cell Mol Life Sci. 2020; 77:2331–42. https://doi.org/10.1007/s00018-019-03405-w PMID:31807814

70. Fafula RV, Paranyak NM, Besedina AS, Vorobets DZ, Iefremova UP, Onufrovych OK, Vorobets ZD. Biological significance of glutathione s-transferases in human sperm cells. J Hum Reprod Sci. 2019; 12:24–28.

https://doi.org/10.4103/jhrs.JHRS_106_18 PMID:31007463

71. Kan HP, Wu FL, Guo WB, Wang YZ, Li JP, Huang YQ, Li SG, Liu JP. Null genotypes of GSTM1 and GSTT1 contribute to male factor infertility risk: a meta- analysis. Fertil Steril. 2013; 99:690–96. https://doi.org/10.1016/j.fertnstert.2012.10.037 PMID:23164539

72. De Marzo AM, Meeker AK, Zha S, Luo J, Nakayama M, Platz EA, Isaacs WB, Nelson WG. Human prostate cancer precursors and pathobiology. Urology. 2003; 62:55–https://doi.org/10.1016/j.urology.2003.09.053 PMID:14607218

73. Sanches LD, Santos SA, Carvalho JR, Jeronimo GD, Favaro WJ, Reis MD, Felisbino SL, Justulin LA Jr. Protective effect of γ-tocopherol-enriched diet on N- methyl-N-nitrosourea-induced epithelial dysplasia in rat ventral prostate. Int J Exp Pathol. 2013; 94:362–72. https://doi.org/10.1111/iep.12042 PMID:24205794

74. Martignano F, Gurioli G, Salvi S, Calistri D, Costantini M, Gunelli R, De Giorgi U, Foca F, Casadio V. GSTP1 methylation and protein expression in prostate cancer: diagnostic implications. Dis Markers. 2016; 2016:4358292. https://doi.org/10.1155/2016/4358292 PMID:27594734

75. Schnekenburger M, Karius T, Diederich M. Regulation of epigenetic traits of the glutathione s-transferase P1 gene: from detoxification toward cancer prevention and diagnosis. Front Pharmacol. 2014; 5:170. https://doi.org/10.3389/fphar.2014.00170 PMID:25076909

76. Knoops B, Goemaere J, Van der Eecken V, Declercq JP. Peroxiredoxin 5: structure, mechanism, and function of the mammalian atypical 2-cys peroxiredoxin. Antioxid Redox Signal. 2011; 15:817–29. https://doi.org/10.1089/ars.2010.3584 PMID:20977338

77. Kim B, Kim YS, Ahn HM, Lee HJ, Jung MK, Jeong HY, Choi DK, Lee JH, Lee SR, Kim JM, Lee DS. Peroxiredoxin 5 overexpression enhances tumorigenicity and correlates with poor prognosis in gastric cancer. Int J Oncol. 2017; 51:298–306. https://doi.org/10.3892/ijo.2017.4013 PMID:28535004

78. Sevilla F, Camejo D, Ortiz-Espín A, Calderón A, Lázaro JJ, Jiménez A. The thioredoxin/peroxiredoxin/ sulfiredoxin system: current overview on its redox function in plants and regulation by reactive oxygen and nitrogen species. J Exp Bot. 2015; 66:2945–55. https://doi.org/10.1093/jxb/erv146 PMID:25873657

79. Veal EA, Underwood ZE, Tomalin LE, Morgan BA, Pillay CS. Hyperoxidation of peroxiredoxins: gain or loss of function? Antioxid Redox Signal. 2018; 28:574–90. https://doi.org/10.1089/ars.2017.7214 PMID:28762774

80. Vance TM, Azabdaftari G, Pop EA, Lee SG, Su LJ, Fontham ET, Bensen JT, Steck SE, Arab L, Mohler JL, Chen MH, Koo SI, Chun OK. Thioredoxin 1 in prostate tissue is associated with gleason score, erythrocyte antioxidant enzyme activity, and dietary antioxidants. Prostate Cancer. 2015; 2015:728046. https://doi.org/10.1155/2015/728046 PMID:26357575

81. Rodriguez-Garcia A, Hevia D, Mayo JC, Gonzalez- Menendez P, Coppo L, Lu J, Holmgren A, Sainz RM. Thioredoxin 1 modulates apoptosis induced by bioactive compounds in prostate cancer cells. Redox Biol. 2017; 12:634–47. https://doi.org/10.1016/j.redox.2017.03.025 PMID:28391184

82. Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, Mueller R, Nolan T, Pfaffl MW, Shipley GL, Vandesompele J, Wittwer CT. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin Chem. 2009; 55:611–22. https://doi.org/10.1373/clinchem.2008.112797 PMID:19246619

83. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods. 2001; 25:402–08. https://doi.org/10.1006/meth.2001.1262 PMID:11846609

84. Gabriel Kuniyoshi ML, Nunes Da Silva-Gomes R, Cavalcante Souza Vieira J, Casemiro Hessel M, Assunção Mareco E, Dos Santos VB, Carvalho RF, De Magalhães Padilha P, Dal-Pai-Silva M. Proteomic analysis of the fast-twitch muscle of pacu (Piaractus mesopotamicus) after prolonged fasting and compensatory growth. Comp Biochem Physiol Part D Genomics Proteomics. 2019; 30:321–332. https://doi.org/10.1016/j.cbd.2019.04.005 PMID:31048267

85. Dionizio AS, Melo CG, Sabino-Arias IT, Ventura TM, Leite AL, Souza SR, Santos EX, Heubel AD, Souza JG, Perles JV, Zanoni JN, Buzalaf MA. Chronic treatment with fluoride affects the jejunum: insights from proteomics and enteric innervation analysis. Sci Rep. 2018; 8:3180. https://doi.org/10.1038/s41598-018-21533-4 PMID:29453425

86. da Silva-Gomes RN, Gabriel Kuniyoshi ML, Oliveira da Silva Duran B, Thomazini Zanella BT, Paccielli Freire P, Gutierrez de Paula T, de Almeida Fantinatti BE, Simões Salomão RA, Carvalho RF, Delazari Santos L, Dal-Pai-Silva M. Prolonged fasting followed by refeeding modifies proteome profile and parvalbumin expression in the fast-twitch muscle of pacu (piaractus mesopotamicus). PLoS One. 2019; 14:e0225864. https://doi.org/10.1371/journal.pone.0225864 PMID:31856193

87. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976; 72:248–54. https://doi.org/10.1006/abio.1976.9999.

PMID:942051

88. Kuleshov MV, Jones MR, Rouillard AD, Fernandez NF, Duan Q, Wang Z, Koplev S, Jenkins SL, Jagodnik KM, Lachmann A, McDermott MG, Monteiro CD, Gundersen GW, Ma'ayan A. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. Nucleic Acids Res. 2016; 44:W90–97. https://doi.org/10.1093/nar/gkw377 PMID:27141961

89. Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, Huerta-Cepas J, Simonovic M, Roth A, Santos A, Tsafou KP, Kuhn M, Bork P, Jensen LJ, von Mering C. STRING v10: protein-protein interaction networks, integrated over the tree of life. Nucleic Acids Res. 2015; 43:D447–52. https://doi.org/10.1093/nar/gku1003 PMID:25352553

90. Jurmeister S, Ramos-Montoya A, Sandi C, Pértega- Gomes N, Wadhwa K, Lamb AD, Dunning MJ, Attig J, Carroll JS, Fryer LG, Felisbino SL, Neal DE. Identification of potential therapeutic targets in prostate cancer through a cross-species approach. EMBO Mol Med. 2018; 10:e8274. https://doi.org/10.15252/emmm.201708274 PMID:29437778

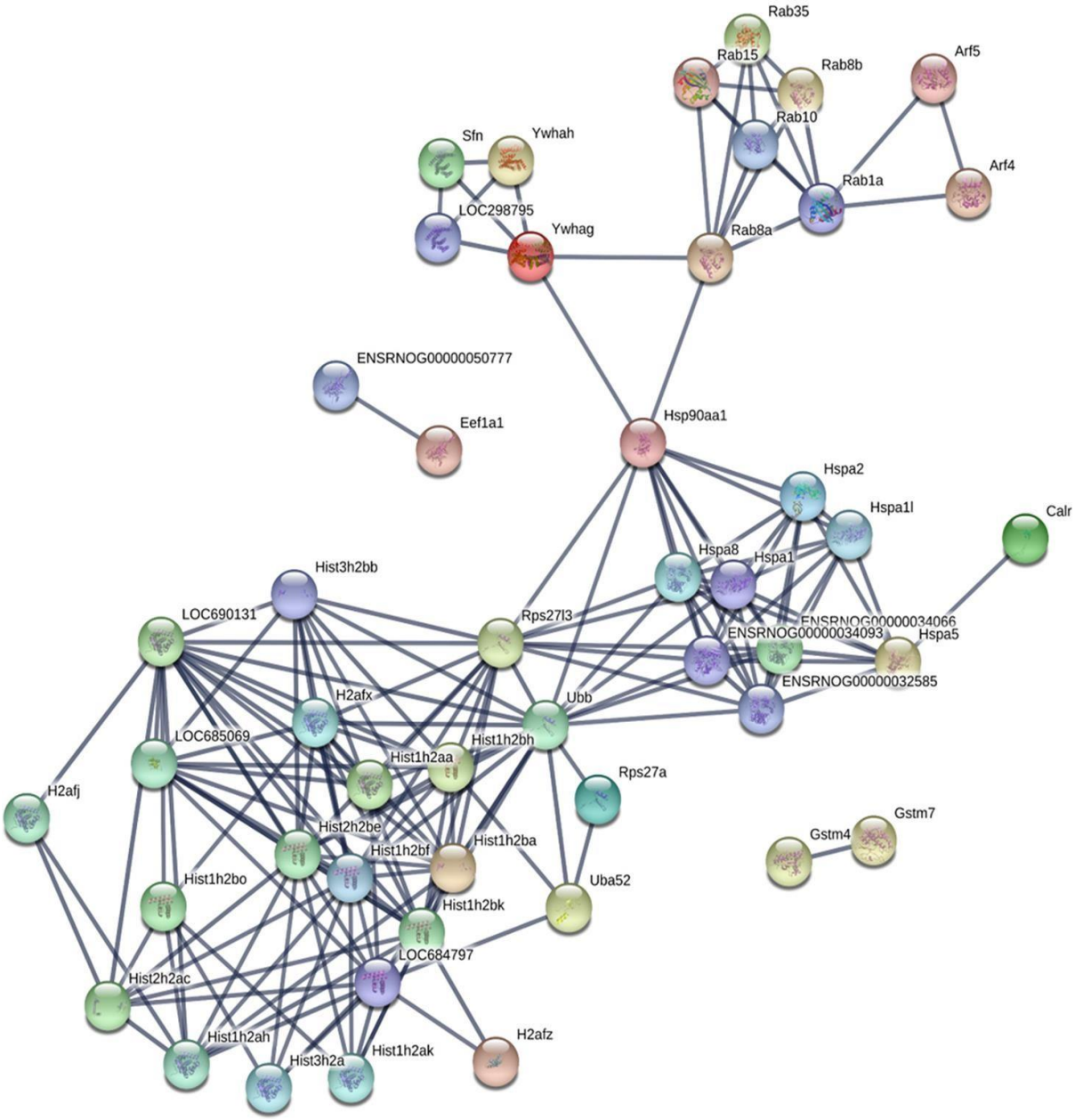
91. Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res. 2017; 45:W98–102. https://doi.org/10.1093/nar/gkx247 PMID:28407145

92. Starruß J, de Back W, Brusch L, Deutsch A. Morpheus: a user-friendly modeling environment for multiscale and multicellular systems biology. Bioinformatics. 2014; 30:1331–32. https://doi.org/10.1093/bioinformatics/btt772 PMID:24443380

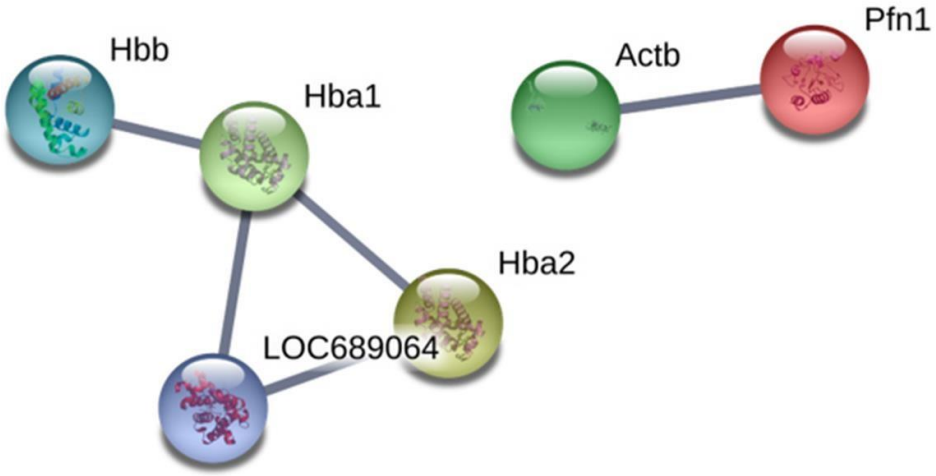
93. Gu Z, Gu L, Eils R, Schlesner M, Brors B. Circlize implements and enhances circular visualization in R. Bioinformatics. 2014; 30:2811–12. https://doi.org/10.1093/bioinformatics/btu393 PMID:24930139

SUPPLEMENTARY MATERIALS

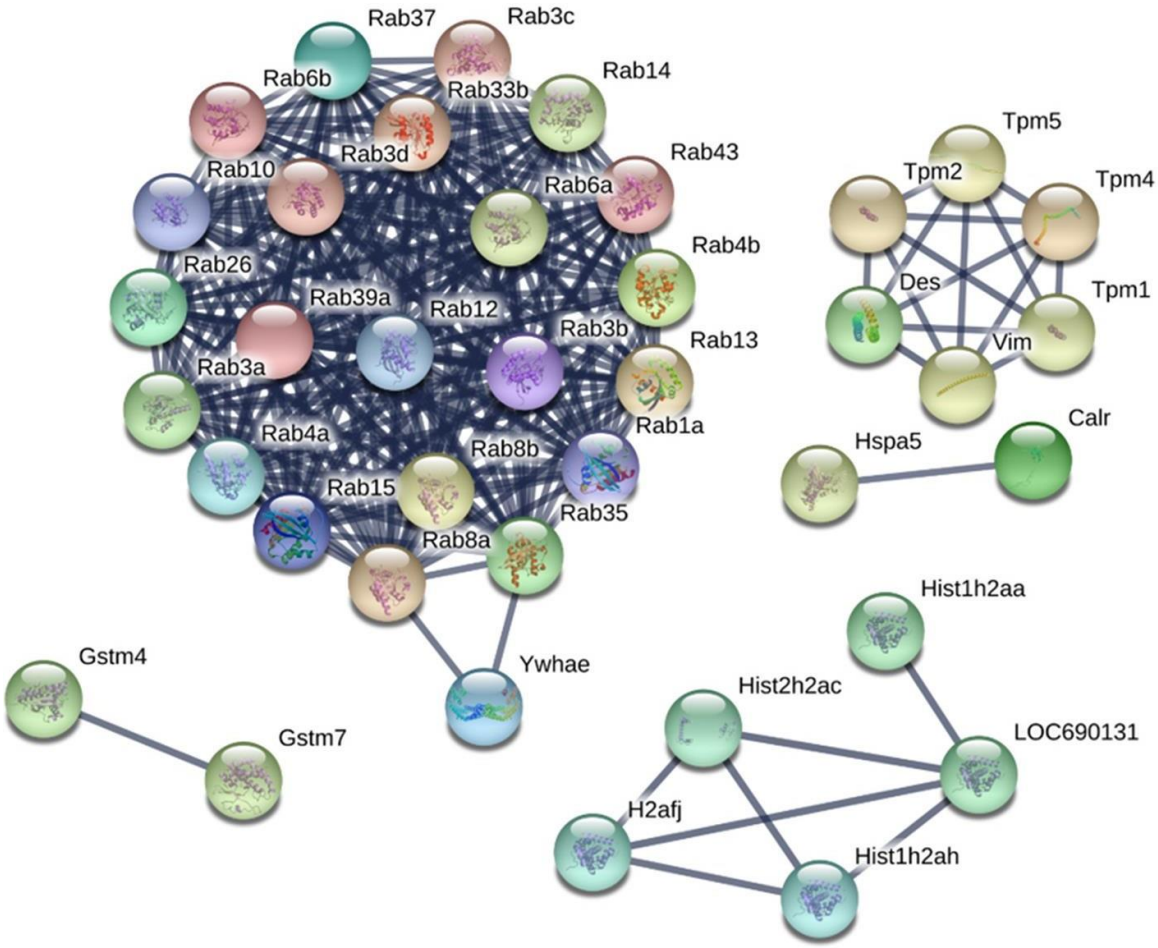
Supplementary Figures



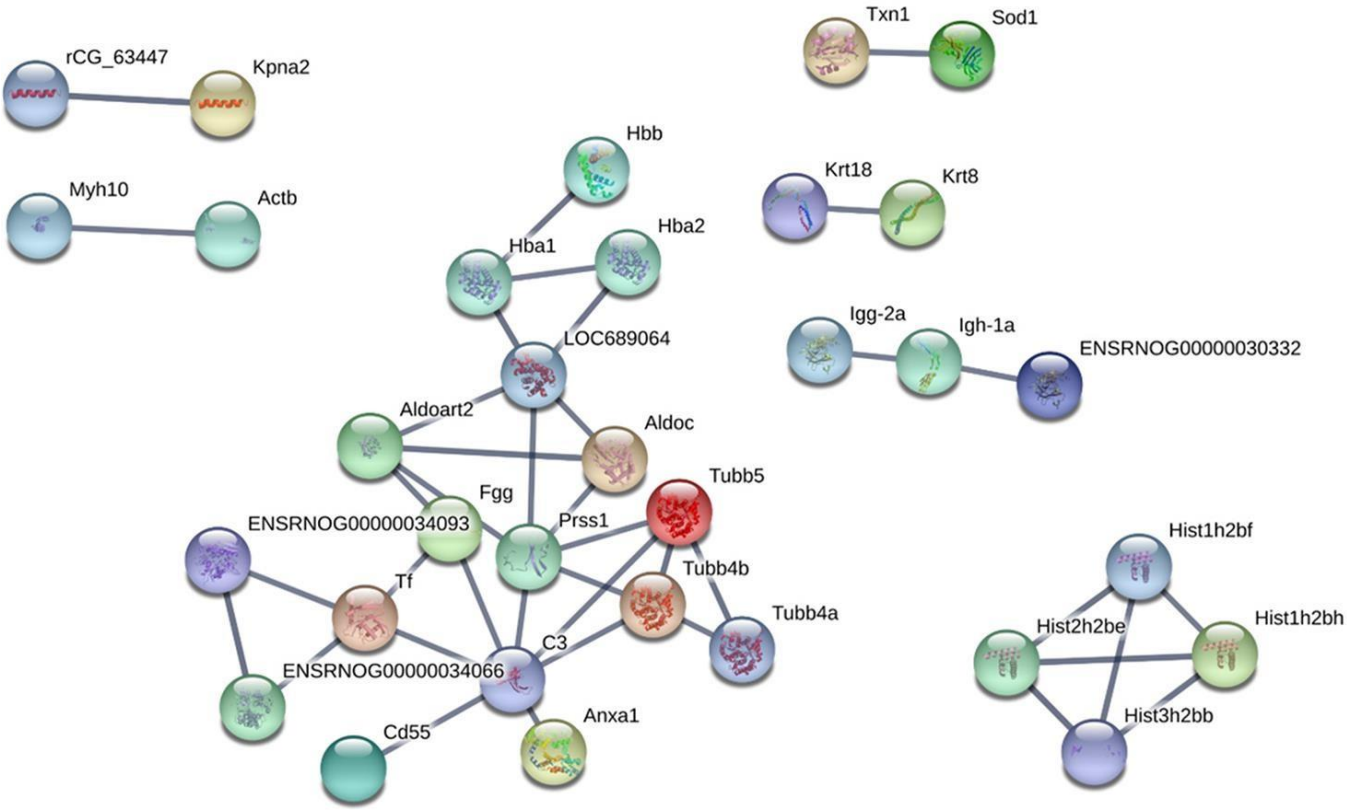
Supplementary Figure 1. Protein-protein interaction network between upregulated proteins on PND 21. Interactions of the identified proteins were mapped by searching the STRING database version 9.0 with a confidence cut-off of 0.7. In the resulting protein association network, proteins are presented as nodes that are connected by lines, whose thickness represents the confidence level (0.7-0.9).



Supplementary Figure 2. Protein-protein interaction network between downregulated proteins on PND 21. Interactions of the identified proteins were mapped by searching the STRING database version 9.0 with a confidence cut-off of 0.7. In the resulting protein association network, proteins are presented as nodes that are connected by lines whose thickness represents the confidence level (0.7-0.9).



Supplementary Figure 3. Protein-protein interaction network between upregulated proteins on PND 540. Interactions of the identified proteins were mapped by searching the STRING database version 9.0 with a confidence cut-off of 0.7. In the resulting protein association network, proteins are presented as nodes that are connected by lines, whose thickness represents the confidence level (0.7-0.9).



Supplementary Figure 4. Protein-protein interaction network between downregulated proteins on PND 540. Interactions of the identified proteins were mapped by searching the STRING database version 9.0 with a confidence cut-off of 0.7. In the resulting protein association network, proteins are presented as nodes that are connected by lines, whose thickness represents the confidence level (0.7-0.9).

Supplementary Table

Supplementary Table 1. Composition of the control (CTR) (AIN-76A) and low protein diet (LPD) (AIN-93).

Ingredients	Normal (CTR) diet (17% of protein) g/Kg	Low protein diet (6% of protein) g/Kg
Casein (84% protein)	202	71.5
Starch	397	480
Dextrin	130.5	159
Sucrose	100	121
L-cystine	3	1
Fiber of pH 101 or pH 102 (microcellulose)	50	50
Soyoil	70	70
Mixtureofvitamins AIN93G*	10	10**
Mixtureofsalts AIN93G*	35	35***
Choline hydrochloride or Choline bitartrate	2.5	2.5

* To know the detailed composition of the salt and vitamin mix, see REEVES et al., 1993. The diet is elaborated by the company PragSoluções (PragSoluções, Jaú, SP, Brazil).

Supplementary Files

Please browse Full Text version to see the data of Supplementary Files 1 and 2.

Supplementary File 1. Total protein identified by mass spectrometry analysis and the list of DEP in the CTR and GLLP groups on PND 21 and 540.

Supplementary File 2. Identification of up and downregulated proteins in the CTR and GLLP groups on PND 21, and 540 that enrichment each molecular term by KOBAs 3.0. Data were presented as p-value, corrected p-value, -Log10 of the p-value, identification, and number of proteins that enriched each molecular term.

Capítulo 4- Anexo

unesp UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus de Botucatu

Instituto de Biociências de Botucatu - Unesp

Certificado

Certificamos que o projeto intitulado "Análises globais integrativas da próstata ventral de ratos submetidos a restrição proteica materna e suas repercussões com o envelhecimento", Protocolo nº 949-CEUA, sob a responsabilidade de Luis Antônio Justulin Junior, que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino) – encontra-se de acordo com os preceitos da Lei nº 11.794, de 9 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi aprovado pela *COMISSÃO DE ÉTICA NO USO DE ANIMAIS* (CEUA), nesta data.

Finalidade:	( ) Ensino	(x) Pesquisa Científica
Vigência do Projeto:	Início: 02/5/2017	Término: 02/5/2019
Espécie/linhagem:	*Rato Sprague Dawley*	
Nº de animais:	*340*	
Peso:	*40-450g*	Idade: 21 a 540 dias
Sexo:	*Macho*	
Origem	*Centro de Bioterismo da Universidade Estadual de Campinas UNICAMP – Campinas/SP*	

Botucatu, 10 de fevereiro de 2017.

Prof. Dr. Bruno Cesar Schimming
Presidente da CEUA

Comissão de Ética no Uso de Animais IBB-Unesp

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