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IDENTIFICAÇÃO E AÇÃO DE RNAs TRANSPORTADOS POR EXOSSOMOS NA ATROFIA DE MIOTUBOS POR TNF-α

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UNIVERSIDADE ESTADUAL PAULISTA
"Júlio de Mesquita Filho"

INSTITUTO DE BIOCIÊNCIAS DE BOTUCATU

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Tese apresentada ao Instituto de Biociências, Campus de Botucatu, UNEP, para obtenção do título de Doutor no Programa de Pós-Graduação em Ciências Biológicas (Genética).

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RESUMO

Os exossomos constituem uma classe de pequenas vesículas extracelulares de aproximadamente 40-150 nm originadas pelo sistema endossomal. Essas vesículas são produzidas constitutivamente por praticamente todos os tipos celulares, e são responsáveis por transportar distintas classes de moléculas, em resposta a diferentes estímulos. O TNF-α é uma citocina pró-inflamatória que em condições tais como AIDS, septicemia, diabetes, insuficiência cardíaca, doença pulmonar obstrutiva crônica, diabetes e câncer, contribui para a atrofia de fibras musculares esqueléticas. Nesse sentido, o objetivo do presente estudo foi caracterizar os RNAs transportados por exossomos de miotubos C2C12 com atrofia induzida por TNF-α e avaliar a ação desses exossomos no fenótipo de mioblastos e miotubos C2C12. Exossomos liberados no meio de cultura de miotubos C2C12 tratados com TNF-α, e de seus respectivos controles, foram isolados por ultracentrifugação e analisados quanto ao seu conteúdo de RNA por RT-qPCR (microRNAs) e sequenciamento de nova geração (RNA total). Além disso, mioblastos e miotubos C2C12 foram tratados com esses mesmos exossomos para analisar a expressão de genes envolvidos com ciclo celular e a capacidade de migração/proliferação (mioblastos), bem como na atrofia celular (miotubos). Nossos resultados demonstraram que exossomos provenientes de miotubos são capazes de alterar os níveis de transcritos de genes envolvidos na proliferação, motilidade e diferenciação de mioblastos em miotubos. Verificamos também que os exossomos provenientes de miotubos tratados com TNFα reduzem a capacidade de migração/proliferação de mioblastos. Além disso, identificamos que o tratamento de miotubos com TNF-α enriquece, nos exossomos, seis miRNAs (mmu-mir-365-3p, -146a-5p, -30b-5p, -34c-3p, -214-3p e -484), e dois RNAs ribossomais mitocondriais (12S e 16S) envolvidos em processos do metabolismo e diferenciação celular. Concluímos que o tratamento de miotubos com TNFα altera RNAs específicos no exossomos, os quais são capazes de alterar a expressão gênica, migração/proliferação e diferenciação de células musculares.

ABSTRACT

Exosomes constitute a class of small extracellular vesicles of approximately 40-150 nm originated by the endosomal system. These vesicles are constitutively produced by virtually all cell types and are responsible for carrying distinct classes of molecules in response to different stimuli. TNF-α is a proinflammatory cytokine that in conditions such as AIDS, septicemia, diabetes, heart failure, chronic obstructive pulmonary disease, diabetes and cancer, contributes to atrophy of skeletal muscle fibers. In this sense, the objective of the present study was to evaluate the expression and action of RNAs transported by exosomes from C2C12 myotubes with TNF-α-induced atrophy in the phenotype of myoblasts and myotubes. Exosomes released in the culture medium from TNF-α treated C2C12 myotubes and from their respective controls were isolated by ultracentrifugation and analyzed for their RNA content by RT-qPCR (microRNAs) and new generation sequencing (total RNA). In addition, myoblasts and C2C12 myotubes were treated with these same exosomes to analyze the expression of genes involved in cell cycle and migration / proliferation capacity (myoblasts), as well as cell atrophy (myotubes). Our results demonstrated that exosomes from myotubes alter the expression genes involved in the proliferation, motility and differentiation of myoblasts into myotubes. We also verified that exosomes from TNFα-treated myotubes decreased migration / proliferation capacity of myoblasts. In addition, we identified that the treatment of myotubes with TNF-α enriches, in the exosomes, six miRNAs (mmu-mir-365-3p, -146a-5p, -30b-5p, -34c-3p, -214-3p and - 484), and two mitochondrial ribosomal RNAs (12S and 16S) involved in cell metabolism and differentiation processes. We conclude that treatment of myotubes with TNFα alters specific RNAs in the exosomes, which, in turn, alter gene expression, migration / proliferation and differentiation of muscle cells.

SUMÁRIO

1. Introdução

1.1. Atrofia do Músculo Esquelético

O músculo esquelético compõe cerca de 40 a 50 por cento da massa corpórea humana sendo constituído por células alongadas, multinucleadas (com núcleos periféricos) denominadas fibras musculares [1]. Além do seu papel notório na atividade locomotora e postural, exerce um importante papel como regulador do metabolismo, atuando diretamente sobre o fígado e o tecido adiposo [2]. O músculo estriado esquelético possui uma elevada capacidade adaptativa em resposta a diferentes condições e estímulos[3]. Por exemplo, exercício físico resistido age no músculo estimulando a síntese de proteínas sarcoméricas, que resulta em hipertrofia das fibra musculares e aumento da força muscular [4,5].

Por outro lado, condições de atrofia, como por exemplo, imobilização, repouso prolongado, desnervação, microgravidade, envelhecimento e doenças crônicas, conduzem o músculo para um processo de perda de volume, com a diminuição na quantidade de proteínas (principalmente proteínas sarcoméricas) das fibras musculares, com consequente perda de força e de resistência à fadiga [6–9]. A perda exacerbada da massa muscular evidenciada em diferentes tipos de doenças crônicas pode resultar num quadro caquético, que inclusive, está correlacionado com um pior prognóstico [10,11].

Estudos tem demonstrado que a atrofia muscular esquelética é conduzida pela ação coordenada de vias moleculares que envolvem IGF1-AKT-FoxO, miostatina, citocinas inflamatórias e o fator transcricional NF-kappa B (NF-kB) [6,12] [para revisão, ver Braun & Gautel 2011 [13]]. Durante a atrofia, os sistemas proteolíticos, com proteínas lisossomais (catepsinas), as calpaínas dependentes de cálcio e o proteassoma dependente de ubiquitina (PDU) atuam na degradação de proteínas musculares [7,8,14]. Estudos em pacientes e em animais têm demonstrado consistentemente que o sistema proteossomal dependente de ubiquitina é um regulador primário da degradação proteica que leva a atrofia muscular[7,12,13,15]. O aumento da expressão de pelo menos uma das 3 enzimas ligases de ubiquitina músculo-específicas MURF1 (*Muscle RING Finger protein 1*), MAFbx (*Muscle Atrophy F-box* ou atrogina-1) [16,17] e E3IIα – é suficiente para induzir a degradação de proteínas miofibrilares em diferentes condições atróficas [7,12,18].

6. Conclusão

Concluímos que o tratamento de miotubos com TNF-α altera a expressão de RNAs específicos no exossomos (miRNAs mmu-mir-365-3p, -146a-5p, -30b-5p, -34c-3p, -214-3p e -484 e os RNAs ribossomais mitocondriais 12S e 16S), os quais são capazes de alterar a expressão gênica, a migração/proliferação e diferenciação de células musculares C2C12 após 12h de tratamento.

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