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**UNIVERSIDADE ESTADUAL PAULISTA
“JÚLIO DE MESQUITA FILHO”
FACULDADE DE MEDICINA**

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**Efeitos de miR-BARTs do vírus de Epstein-Barr na
modulação de vias de sinalização intracelular em
células derivadas de linfomas humanos**

Tese apresentada à Faculdade de Medicina,
Universidade Estadual Paulista “Júlio de
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Orientador: Prof. Dr. Deilson Elgui de Oliveira

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Effects of Epstein-Barr virus miR-BARTs in modulating
intracellular pathways in human lymphoma cells

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Thesis Supervisor: Deilson Elgui de Oliveira, Ph.D.

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“Science, my lad, is made up of mistakes, but they are mistakes which it is useful to make because they lead little by little to the truth.”

Jules Verne, A Journey to the Centre of the Earth.

“We’re forever teetering on the brink of the unknowable and trying to understand what can’t be understood. It’s what makes us men.”

Isaac Asimov, The Caves of Steel

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List of Abbreviations

4-HT	4-hydroxytamoxifen
AmBic	Ammonium bicarbonate buffer
BCR	B cell receptor
BL	Burkitt lymphoma
CBP	CREB-binding protein
CDT1	Chromatin licensing and DNA replication factor 1
cHL	Classic Hodgkin lymphoma
Cp	C-latency promoter
CR2 or CD21	Complement receptor 2
DLBCL	Diffuse large B cell lymphoma
DOX	doxycycline
DS	Dyad symmetry
DTT	Dithiothreitol
EBNAs	EBV-encoded nuclear antigens
EBV	Epstein-Barr virus
ESCRT	Cellular endosomal sorting complexes required for transport
FBS	Fetal bovine serum
FDR	False discovery rate
FR	Family of repeats
GC	Gastric carcinoma
gp350	Glycoprotein 350
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HPV	Human papillomavirus
IA-LPD	Immunodeficiency-associated lymphoproliferative disorders
IAA	2-iodoacetamide
IgA	Immunoglobulin A
IM	Infectious mononucleosis

LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LCV	Lymphocryptovirus
LG	Lymphomatous granulomatosis
LMPs	Latent membrane proteins
MAPKs	MAP kinases
MCD	Multicentric Castleman's disease
MHC class II	Major histocompatibility complex class II
miRNAs	Micro-RNAs
NaB	Sodium butyrate
NPC	Nasopharyngeal carcinoma
ORC2	Origin recognition complex subunit 2
ORF	Open Reading Frame
OriP	Replication origin region
PBL	Plasmablastic lymphoma
PEL	Primary effusion lymphomas
PI3K	Phosphatidylinositol 3 kinase
PL-HIV	People living with HIV
pIgR	Polymeric immunoglobulin receptor
PTLD	Post-transplant lymphoproliferative disorders
RBP	RNA binding protein
Rp	Rta promoter
sncRNAs	Small non-coding RNAs
STR	Short tandem repeat
T7EI	T7 endonuclease I
TCF-3	Transcription factor 3
TFA	Trifluoroacetic acid
TFIIA	Transcription factor IIA
TIDE	Track of indels by decomposition
TPA	Tetradecanoylphorbol-13-acetate
VCRs	Viral replication compartments
VRC	Viral replication compartments

VZV	Varicella-Zoster virus
Wp	W promoter
WT	Wilde type
Zp	Zta promoter
ZRE	Z-responsive elements

Resumo

O linfoma de Burkitt (LB) é um linfoma não-Hodgkin de células B altamente agressivo, associado à infecção pelo vírus de Epstein-Barr (EBV) em virtualmente todos os casos de forma endêmica da doença. O EBV foi o primeiro vírus humano descoberto codificar miRNAs no genoma viral, agrupados em duas regiões distintas: *BamHI fragment H rightward open reading frame 1* (miR-BHRFs) e *BamHI-A rightward transcripts* (miR-BARTs). Embora extensivamente estudado no contexto de neoplasias epiteliais, o papel dos EBV miR-BARTs na patogênese de linfomas associados à infecção pelo EBV é essencialmente desconhecido. Este estudo tem como objetivo investigar os efeitos da supressão dos EBV miR-BART7 e miR-BART9 em células Akata EBV-positivas através da edição dirigida do genoma viral por CRISPR/Cas9. Ambos os mutantes gerados apresentaram baixos níveis de expressão dos miRNAs virais, demonstrando a eficácia da edição mediada por CRISPR/Cas9. A supressão de ambos miRNAs nos mutantes acarretou em uma diminuição drástica de viabilidade e proliferação celular, levando ao aumento da expressão de genes líticos virais. A análise do perfil proteômico dos mutantes demonstrou que a supressão do EBV miR-BART7 aumentou a expressão de diversas proteínas de ligação de RNA, enquanto a supressão de miR-BART9 levou ao aumento da expressão de DNA topoisomerasas e proteínas associadas a via ubiquitina-proteossoma. Nossos resultados demonstram importantes papéis e mecanismos associados a expressão de EBV miR-BART7 e miR-BART9 no aumento da proliferação celular e manutenção da latência viral, sequestrando vias celulares importantes associadas ao ciclo lítico viral.

Palavras-chave: Vírus de Epstein-Barr, linfoma de Burkitt, microRNAs virais, ebv-miR-BART7, ebv-miR-BART9, CRISPR/Cas9, edição gênica.

Abstract

Burkitt Lymphoma (BL) is a highly aggressive B-cell non-Hodgkin lymphoma associated with the Epstein-Barr virus (EBV) infection in virtually all cases of its endemic African form. EBV was the first human virus found to encode viral miRNAs, clustered in two distinct regions of the viral genome: the BamHI fragment H rightward open reading frame 1 (miR-BHRFs) and the BamHI-A rightward transcripts (miR-BARTs). Although extensively studied in epithelial cancers, the role of EBV miR-BARTs on the pathogenesis of EBV-associated lymphomas is essentially unknown. Therefore, this study sought to investigate the effects of EBV miRs BART7 and BART9 suppression in Akata EBV-positive cells using CRISPR/Cas9-targeted mutagenesis. Overall, both Akata mutants harboring the edited EBV genomes exhibited low levels of viral miRNAs expression, demonstrating the efficacy of the CRISPR/Cas9-mediated knockdown of EBV miR-BART7 and 9. A reduction in cell viability, proliferation, and increased expression of viral lytic genes was found in both mutants. The knockdown of EBV miR-BART7 significantly increased the expression of several RNA binding proteins (RBPs), while the knockdown of EBV miR-BART9 increased the expression of DNA topoisomerases and ubiquitin/proteasome proteins. Our results unravel potential roles for EBV miR-BART7 and miR-BART9 increasing cell proliferation and maintaining viral latency by hijacking critical cellular pathways associated with the viral lytic cycle.

Keywords: Epstein-Barr Virus, Burkitt lymphoma, viral microRNAs, ebv-miR-BART7, ebv-miR-BART9, CRISPR/Cas9, knocking down.

7 References

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