ABSTRACT

The Epstein-Barr virus (EBV)-encoded BARF1 (BamHI-A rightward frame-1) is expressed in EBV-positive malignancies such as nasopharyngeal carcinoma, EBV-associated gastric carcinoma, B cell lymphoma and nasal NK/T-cell lymphoma, and has been shown to have important role in oncogenesis. However, the mechanism by which BARF1 elicits its biological effects on EBV-positive malignant cells is unclear. In this study, the effects of BARF1 gene silencing on cell proliferation and apoptosis were investigated in EBV-positive malignant cells. We observed that silencing of BARF1 using RNAi significantly inhibits cell proliferation and induces apoptosis-mediated cell death by collapsing the mitochondrial membrane potential in AG876 and Hone-Akata cells. BARF1 knockdown also upregulates the expression of pro-apoptotic proteins and downregulates the expression of anti-apoptotic proteins. In BARF1-downregulated cells, the Bcl-2/BAX ratio is decreased. The caspase inhibitor z-VAD-fmk was found to rescue siBARF1-induced apoptosis in these cells. Immunoblot analysis showed significant increased levels of cleaved caspase 3 and caspase 9. Using western blotting analysis, we also observed a significant increase in cytochrome c level in the cytosolic fractions of BARF1-depleted cells. Immunoprecipitation and further immunoblot analysis revealed that depletion of BARF1 activated formation of apoptosome complex in BARF1-silenced cells. In conclusion, siRNA-mediated BARF1 downregulation induced caspase-dependent apoptosis via the mitochondrial pathway through modulation of Bcl-2/BAX ratio in AG876 and Hone-Akata cells. Targeting BARF1 using siRNA has potential to be developed as a novel therapeutic strategy in treatment of EBV-associated malignancies.

ABSTRAK

BARF1 (BamHI-A rightward frame-1) yang dikodkan oleh Epstein -Barr virus (EBV) diekspresikan di dalam malignansi positif EBV seperti karsinoma nasofarinks, karsinoma gastrik positif EBV, limfoma sel B dan limfoma sel NK/T hidung, telah terbukti mempunyai peranan yang penting dalam proses pembentukan kanser. Walau bagaimanapun, mekanisme dengan mana BARF1 menimbulkan kesan biologi pada sel-sel malignan positif EBV adalah tidak jelas. Dalam kajian ini, kesan gen BARF1 ke atas percambahan sel dan apoptosis telah disiasat dalam sel-sel malignan EBV positif. Kami mendapati bahawa pembungkaman BARF1 menggunakan RNAi dapat membendung percambahan sel dan mendorong sel mati secara apoptosis dengan meruntuhkan potensi membran mitokondria dalam sel- sel AG876 dan Hone-Akata dengan ketara. Pembungkaman BARF1 juga meningkatkan ekspresi protein pro-apoptosis dan menurunkan ekspresi protein anti-apoptosis. Di dalam sel-sel di mana BARF1 disenyapkan, nisbah Bcl-2/BAX berkurangan. Di dalam sel-sel ini juga, perencat caspase z-VAD-fmk didapati dapat menyelamatkan apoptosis yang disebabkan oleh siBARF1. Analisis blot imun menunjukkan peningkatan ‘cleaved’ caspase 3 dan 9 pada tahap signifikan. Menggunakan analisis ‘Western blot’, kami juga memerhatikan peningkatan yang ketara bagi tahap cytochrome c dalam pecahan sitosol sel-sel yang kehabisan BARF1. Kesimpulannya, pengurangan BARF1 yang disebabkan oleh siRNA menyebabkan apoptosis yang mana bergantung kepada caspase melalui laluan mitokondria secara modulasi nisbah Bcl-2/BAX di dalam sel-sel AG876 dan Hone-Akata. Pensasaran BARF1 menggunakan siRNA mempunyai potensi untuk dibangunkan sebagai strategi terapeutik novel dalam rawatan malignansi yang berkait dengan EBV.

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List of symbols and abbreviations

× g	relative centrifugal force
°C	degree Celcius
µg	microgram
µl	microlitre
ADCC	antibody-dependent cellular cytotoxity
AIDS	acquired immunodeficiency syndrome
Apaf-1	Apoptotic protease-activating factor-1
ASLV	Avian sarcoma leucosis virus
ATP	adenosine triphosphate
Bak	Bcl-2 homologous antagonist killer
BARF1	BamH1-A Reading Frame-1
BAX	Bcl-2-associated X
Bcl-2	B-cell CLL/lymphoma 2
BH	Bcl-2 homology
Bid	BH3 domain-containing proapoptotic Bcl-2 family protein
bp	base pairs
CAD	caspase-activated deoxyribonuclease
cDNA	complementary DNA
CD21	cluster of differentiation 21
CD95	cluster of differentiation 95
cIAP-2	cellular inhibitor of apoptosis 2
CO_2	carbon dioxide
COX IV	cytochrome c oxidase IV
CSF	colony stimulating factor
CTL	cytotoxic T lymphocyte
Cyto C	cytochrome c
dATP	deoxyadenosine triphosphate
DISC	death-inducing signaling complex
DNA	deoxyribonuclease acid
dNTP	deoxyribonucleotide triphosphate
dsRBD	dsRNA-binding domain
dsRNA	double-stranded RNA
DTT	Dithiothreitol

EBERs	EBV-encoded RNAs
EBNA	Epstein-Barr virus nuclear antigen
EBNA-LP	Epstein-Barr virus nuclear antigen leader protein
EBV	Epstein-Barr virus
EDTA	ethylenediaminetetraacetic acid
FADD	Fas-associated death domain
FasL	Fas ligand
FITC	fluoresceine-isothiocyanate
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
GC	gastric carcinoma
h	hour
HCV	Hepatitis C virus
HIV-1	human immunodeficiency virus 1
HLA	human leukocyte antigen
HPV	human papillomavirus
H-Ras	Harvey rat sarcoma viral oncogene homolog
ICAD	inhibitor of caspase-activated deoxyribonuclease
IFN	interferon
IgG	immunoglobulin G
IL	interleukin
IM	infectious mononucleosis
IP	immunoprecipitation
JC-1	mitochondrial membrane potential assay reagent
LB	Luria Bertani
LMP1	Latent Membrane Protein-1
LTR	long terminal repeat
M	molar
MAPK	mitogen-activated protein kinase
MHC	major histocompatibility complex
min	minute
miRNA	microRNA
ml	millilitre
MMP	mitochondrial membrane potential ($\Delta\Psi$)
mRNA	messenger RNA
NF- κB	nuclear factor kappa-light-chain enhancer of activated B cells

NK	natural killer
nm	nanometer
NPC	nasopharyngeal carcinoma
ORF	open reading frame
PARP	poly-(ADP-ribose) polymerase
PATAS	patas monkey kidney cells
PAZ domain	PIWI–Argonaute–Zwille domain
PBS	phosphate-buffered saline
PI	propidium iodide
PTGS	posttranscriptional gene silencing
qPCR	quantitative polymerase chain reaction
RISC	RNA induced silencing complex
RNAi	RNA interference
rpm	revolutions per minute
RPMI	Roswell Park Memorial Institute medium
s	second
SARS-CoV	SARS-associated coronavirus
SD	standard deviation
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
siRNA	small interfering RNAs
TBS	tris-buffered saline
TRAF	tumor necrosis factor receptor-associated factor
UTR	untranslated region
UV	ultraviolet
w/v	weight/volume
WST-1	water soluble tetrazolium salt-1
z-VAD-fmk	caspase inhibitor VI

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