Original Article | Iran J Med Microbiol. 2024; 18(1): 16-24

10.30699/ijmm.18.1.16

Iranian Journal of Medical Microbiology | ISSN:2345-4342

Exploring *EBNA1*-Mediated Regulation of Key Cellular Genes in Glioblastoma Multiforme: Implications for EBV-Associated Pathogenesis

Amir Hossein Alipour[1,2,3], Hamideh Najafi[1], Zahra Ziafati Kafi[1], Seyed Mohammad Ali Hashemi[3], Jamal Sarvari[3], Arash Ghalyanchi Langeroudi[1*]

1. *Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*
2. *Gene Therapy Research Center, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran*
3. *Department of Bacteriology and Virology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*

ABSTRACT

Background and Aim: Infection with Epstein-Barr virus (EBV) ranks as one of the most substantial risk factors associated with Glioblastoma multiforme (GBM). At the core of this intricate relationship lies the EBV nuclear antigen-1 (*EBNA1*) protein, a central figure with a remarkable ability to regulate the expression of both cellular and viral genes. This research delves into the impact of *EBNA1* on the expression patterns of four cellular genes - *MDMX*, *MDM2*, *MYC*, and *BIRC5* in the U87MG cell line.

Materials and Methods: We divided U87MG cells into two distinct groups. The first group involved cells that were transfected with a plasmid containing the *EBNA1* gene, while the second group consisted of cells that were transfected with a control plasmid. To evaluate the transcriptional activity of *MDMX*, *MDM2*, *MYC*, and *BIRC5* genes in both sets of cells, we employed a real-time PCR technique. Any observed differences were considered statistically significant if the associated P-values were less than 0.05.

Results: Our findings demonstrated a substantial three-fold increase in the expression of the *MDMX* gene when U87MG cells were transfected with *EBNA1* plasmid (*P*=0.02). Although the cells transfected with *EBNA1* plasmid displayed great elevations in the expression levels of *MDM2*, *MYC*, and *BIRC5* genes, these alterations were not statistically significant.

Conclusion: The outcomes of this investigation have unveiled that *EBNA1* has the ability to trigger the expression of four crucial cellular genes, which wield substantial influence in the genesis of GBM within glioblastoma astrocytoma cells. This underscores the potential impact of *EBNA1* on the evolution of GBM, particularly in individuals harboring EBV.

Keywords: *BIRC5*, *EBNA1*, Epstein–Barr virus, Glioblastoma, *MDMX*, *MDM2*, *MYC*

Received: 2023/12/05; **Accepted**: 2024/02/16; **Published Online:** 2024/03/18

Corresponding Information: **Arash Ghalyanchi Langeroudi**, *Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran* Email: ghalyana@ut.ac.ir

1. Introduction

Glioblastoma, formerly referred to as glioblastoma multiforme (GBM), stands as one of the most aggressive forms of brain cancer, marked by symptoms including headaches, personality alterations, nausea, and stroke-like manifestations **(1)**. The onset of these symptoms is typically rapid and can progress to unconsciousness **(1)**. While the exact causes of GBM and other gliomas remain elusive, there is a documented heightened risk in a small subset of GBM patients exposed to ionizing radiation, chemical agents, or genetic predispositions **(2)**. Recently, greater attention has been focused on the potential viral origins of gliomas, as they may act as oncomodulators, with viral proteins capable of augmenting neoplastic processes through the disruption of various intracellular signaling pathways

(3, 4). Epstein-Barr virus (EBV) is alternatively known as HHV-4 and holds the distinction of being the first recognized human oncovirus **(5)**. While B-cells in the bone marrow are commonly considered the primary latent reservoir of EBV, their presence has also been detected within the brain **(7)**. Several central nervous system (CNS) disorders, such as acute encephalitis, acute cerebellar ataxia, demyelinating diseases, myelitis or meningitis, and other CNS neuropathies, have been suggested to have potential links to EBV infection **(8)**. Complement receptor 2 (CR2), recognized as the primary cellular receptor for Epstein-Barr virus (EBV), has been identified in astrocytes, facilitating the virus's entry into astrocyte cell lines and subsequently promoting increased proliferation **(9, 10)**. Significantly, EBV is frequently detected in primary CNS lymphomas, particularly diffuse large B-cell lymphomas and lymphoid granulomatosis **(11)**. Within the realm of EBV proteins, EBV nuclear antigen-1 (*EBNA1*) stands out as the lone protein found across all EBV latency types **(12)**. *EBNA1* plays a pivotal role in the regulation of transcription for both viral and cellular promoters **(12)**. Functioning as a DNA-binding transcription factor, this protein interacts with diverse DNA regions within the cellular genome, including the promoters of genes whose transcription is under the influence of *EBNA1* **(13, 14)**.

In this study, the first pair of cellular genes scrutinized were mouse double minute X homolog (*MDMX*) and MDM2. These genes produce proteins that are capable of binding to the p53 tumor suppressor protein, effectively inhibiting its function. Notably, both MDMX and MDM2 have been observed to be overexpressed in numerous human cancer types **(15)**. However, it's important to distinguish their mechanisms: MDM2, which is a nuclear-localized E3 ubiquitin ligase, directly degrades p53, whereas the MDMX protein inhibits p53 by binding to its transcriptional activation domain. Additionally, MDMX forms an interaction with MDM2 through the RING finger domain, preventing the degradation of the latter **(16)**. On a different note, MYC, functioning as a transcription factor, exerts influence over a range of biological processes, including cell growth, proliferation, apoptosis, and cellular metabolism **(17)**. Meanwhile, *BIRC5*, by interfering with caspase activity, mitigates cell death, and thus, its elevated expression has been linked to various cancer types **(18, 19)**.

Building upon the diverse characteristics of *EBV-EBNA1*, our novel research endeavored to assess the impact of *EBNA1* on the expression profiles of four critical cellular oncogenes associated with glioblastoma (GBM). Specifically, we focused on *MDMX*, *MDM2*, *MYC*, and *BIRC5*. To unravel this intricate interplay, we employed the U87MG glioblastoma astrocytoma cell line, which has been genetically modified through transfection with a plasmid carrying the *EBNA1* gene.

2. Materials and Methods

Plasmid Preparation and Validation, and Bacterial Transformation

In this research endeavor, we utilized the pCEP4 plasmid (Thermo Fisher Scientific, USA), which is an EBV-based plasmid containing the *EBNA1* gene alongside the hygromycin B resistance gene, in addition to pcDNA3.1/hygro as control plasmid devoid of the *EBNA1* gene which was obtained from the Iranian Biological Resource Center (IBRC). These plasmids were individually transformed and propagated within the *Escherichia coli DH5α* strain. To confirm the presence of the *EBNA1* gene in the pCEP4 plasmid, we conducted enzyme digestion and colony PCR. Subsequently, both the recombinant and control plasmids were separately extracted utilizing the MBST plasmid isolation kit (Molecular Biological System Transfer, Tehran, Iran), and their quality and concentrations were assessed through gel electrophoresis and spectrophotometry.

Cell Culturing, Transfection, and Selection of Transfected Cells

U87MG cells, a widely employed human glioblastoma cell line in brain cancer research, were acquired from the IBRC and cultivated in Dulbecco's Modified Eagle Medium (DMEM; Bioidea, Tehran, Iran) supplemented with 10% Fetal Bovine Serum (FBS; Gibco, USA) under standard conditions of 37°C and 5% CO_2 within a 6-well plate. Once the cells reached an approximate confluency of 80%, they were subjected to transfection using an optimized concentration of plasmids (4 µg) and DNA-fectamine (6 µL; BioBasic, Canada). One set of cells received the recombinant plasmid carrying the *EBNA1* gene, while the other group was transfected with a mock plasmid lacking the *EBNA1* gene. Following transfection, both cell populations underwent selection using 500 µg/mL hygromycin B (BioBasic, Canada). To attain complete cell selection, transfected cells were maintained under stable hygromycin B concentrations over several passages for approximately 25 days.

RNA Extraction, cDNA Synthesis, Real-time PCR, and Data Analysis

The total RNA from both cell groups was extracted using an RNA Isolation Kit (Dena Zist, Iran). The quantity and quality of the RNA from each group were assessed through spectrophotometry and electrophoresis, respectively. To ensure the exclusion of plasmid contamination, RNase-free DNase (Sinaclon, Tehran, Iran) was employed. Subsequently, a cDNA Synthesis Kit

(Dena Zist, Iran) was utilized to reverse-transcribe the extracted RNA from each sample into stable and optimized concentrations of cDNA. Real-time PCR based on SYBR green fluorescence was then conducted using specific primers to measure the expression of the chosen cellular genes. For relative quantification of gene expression, the beta-actin gene was used as a reference gene. In each 20 µL final volume mixture, there were 10 µL of 2x SYBR Green Master Mix (Ampliqon Inc., Denmark), 7 µL of molecular grade water, 0.5 µL of each specific primer pair (refer to Table 1), and 2 µL of cDNA. Subsequently, the qRT-PCR was conducted on an ABI 7500 instrument with an optimized thermal cycling protocol (Table 2). All cycle threshold (Ct) values were standardized, and then, utilizing Microsoft Excel, the normalized values were computed through the application of the $2^{-\Delta\Delta CT}$ method. Statistical comparisons of means were performed using the Mann–Whitney U test. A significance threshold of P-value<0.05 was adopted for statistical significance.

Table 1. Primers employed for assessing gene expression via quantitative real-time PCR

Gene Name	Sequence	Product size	Reference
MDMX	5′-GCCTGCCTTGGTGGTT-3′ 5′-CCTAACTGCTCTGATACTGACTC-3′	160 bp	(20)
MDM2	5′-AACCACCTCACAGATTCCA-3′ 5′-GCACCAACAGACTTTAATAACTTC-3′	87 bp	(21)
MYC	5′-TCACACCCTTCTCCCTT-3′ 5′-CGCTCCACATACAGTCC-3′	180 bp	(22)
BIRC5	5′-AGTTGGAGTGGAGTCTGG-3′ 5′-CTTGCTGGTCTCTTCTGG-3′	144 bp	(22)
EBNA1	5′-GGGTGGTTTGGAAAGCATCG-3′ 5′-CTTACTACCTCCATATACGAACACA-3′	156 bp	(22)
Beta-actin	5′-GCCTTTGCCGATCCGC-3′ 5′-GCCGTAGCCGTTGTCG-3′	90 bp	(21)

Table 2. Thermal cycling protocol for quantitative real-time PCR reaction

Steps	Time	Cycle	Temperature
Initial denaturation	15 minutes	1X	95°C
Denaturation	15 seconds	40X	95°C
Annealing/Extension	1 minute		62°C

3. Results

Expression Profiling of Selected Genes Following *EBNA1* Transfection

The study assessed the expression of the *MDMX* gene in U87MG cells that were transfected with *EBNA1* in comparison to control cells containing a mock plasmid, as shown in Figure 1. Analysis of real-time PCR data indicated that the presence of *EBV-EBNA1* led to an approximately threefold increase in the expression of the *MDMX* gene (*P*=0.02). In Figure 1, we also present a comparison of *MDM2*, *MYC*, and *BIRC5* gene expression levels in cells transfected with *EBNA1* and those transfected with a control plasmid. Notably, U87MG cells containing *EBNA1* exhibited a higher expression of these three genes compared to cells with the control plasmid.

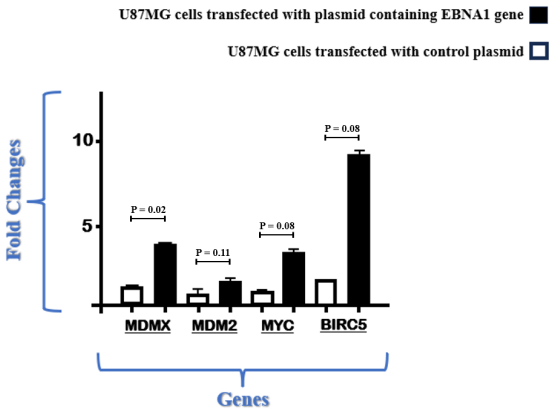

Figure 1. Alterations in the expression of *MDMX*, *MDM2*, *MYC*, and *BIRC5* detected through real-time PCR analysis

4. Discussion

Today, it is abundantly clear that viral infections contribute to the development of cancer **(23)**. EBV is among carcinogenic viruses whose role in causing epithelial cancers (such as nasopharyngeal carcinoma and gastric cancer) as well as lymphoid cancers (like Burkitt's lymphoma, Hodgkin's lymphoma, etc.) has been fully proven **(24, 25)**. Although there is a small probability, EBV infection can be seen in the central nervous system (CNS), especially in immunocompromised people **(7)**. In a study by Gaffari *et al.*, it was revealed that the EBV genome was detected in 21.4% of samples from GBM patients but not in control brain tissue specimens. Among these positive cases, 66% were identified as having EBV type 1, 11% as EBV type 2, and 22% as dual positive for both EBV types 1 and 2 **(26)**. Additionally, Fonseca *et al.* conducted a study using conventional PCR to analyze 75 frozen glioma samples, reporting an incidence of EBV DNA at 14.7% in WHO grade III and WHO grade IV glioma samples **(27)**.

EBV contributes to carcinogenesis through a range of its distinct proteins, and one of these key proteins is EBNA1, which is expressed across all EBV latency types **(12)**. Although EBV-EBNA1 is not yet classified as a direct oncogene, its multifaceted roles have a significant impact. This includes interactions with various cellular proteins, leading to alterations in their functions **(28, 29)**, binding to diverse RNAs **(30)**, and even binding to specific DNA sequences within the host cell's genome. These combined actions make EBNA1 a potent driver of cellular oncogenesis **(28, 31)**. Numerous investigations have demonstrated that this protein can alter the expression of cellular genes by interacting with their promoters **(32)**, and if these affected genes were cellular oncogene types, the host's condition would be highly critical. In our prior research endeavor, we demonstrated that *EBNA1* can bind to the promoters of other viral genes and influence their expression **(22)**. Consequently, this highlights that EBV, beyond its mechanisms associated with virulence, possesses the ability to entirely reshape the dynamics of other viral infections within the host, contributing to a heightened exacerbation of the patient's condition.

Our present study revealed that in U87MG cells transfected with an *EBNA1*-containing plasmid, there was a notable increase in the mRNA expression of *MDMX* and *MDM2* cellular genes when compared to the control cell group. *MDMX* and *MDM2* belong to the MDM family, which is composed of these two proteins and their derivatives **(33)**. *MDMX* and *MDM2* play critical roles in the negative regulation of the p53 tumor suppressor, which is required for synchronized malignancy suppression and maintaining cells **(16)**. Numerous investigations have shown that *MDMX* and *MDM2* were overexpressed in a variety of human tumors, including retinoblastoma, breast cancer, lung cancer, colon cancer, and glioblastoma **(34-36)**. It has been shown that approximately 80% of gliomas have a p53 pathway malfunction, and 40% of gliomas show a p53 mutation or deletion **(37)**, which may be related to *MDMX* and *MDM2* overexpression. Based on the comprehensive assessment which was conducted by Van Meir et al. it was shown that changes in the p53 pathway are important in the development of GBM. Surprisingly, genomic analysis of human GBM genes and their critical pathways revealed that p53 signaling was changed in 87% of GBM cases **(38)**. As part of a study conducted by the American Association for Cancer Research (AACR), it was revealed that *MDMX* underwent alterations in 5.26% of GBM patients **(39)**. Additionally, a separate investigation led by Riemenschneider and colleagues identified a 5- to 25-fold overexpression of *MDMX* in 2.4% of 208 cases of glioma **(40)**. In another investigation by Arjona *et al.*, *MDMX* was shown to be overexpressed in 27% of 86 GBM samples. Moreover, this study revealed a noteworthy increase in the abundance of *MDMX* in low-grade astrocytic tumors, indicating that this phenomenon might signify an initial event in the progression of carcinogenesis **(41)**. MDMX does not possess E3 ligase activity, and as a result, one of its methods for reducing P53 levels involves forming a heterodimer with *MDM2* to activate *MDM2*'s ubiquitination function. This event is commonly referred to as the p53-*MDM2*/*MDMX* loop, in which both *MDM2* and *MDMX* jointly restrain the tumor suppressor function of p53 **(42)**. As mentioned, MDM2 plays an imperative role in down-regulating p53 activity via ubiquitin-dependent degradation **(42)**. Additionally, several studies revealed that the p53-ARF-MDM2 pathway was dysregulated in a substantial majority of GBM patients and GBM cell lines **(43, 44)**. In this regard and in agreement with us, Werner *et al.* showed that *MDM2* was overexpressed in 14% of GBM cases (38). Moreover, according to Reifenberger *et al.*, 8–10% of GBM had MDM2 overexpression **(45)**.

Our research findings revealed a notable threefold increase in the expression of the *MYC* gene in U87MG cells that were transfected with the *EBNA1* plasmid when compared to cells transfected with the control plasmid. The great majority of malignancies have unregulated activity of this cellular oncogene, which is controlled as a downstream effector by cell metabolism **(46)**. It has been shown that the MYC protein plays a pivotal role in the development and progress of GBM **(47)**. MYC overexpression has been reported to correlate with a higher grade of malignancy in glioma **(48, 49)**. *MYC* is in charge of EGFR overexpression at the transcriptional level **(50)** as well as the expression or transcriptional suppression of some miRs involved in glioma chemoresistance **(51,**

52). Some MYC tumorigenic effects in GBM are exerted through the molecular partners belonging to its protein network **(53, 54)**. Moreover, MYC is one of the master genes controlling the stem cell characteristics of cancer-initiating glioblastoma stem cells (GSCs) **(55)**. Furthermore, our prior research showcased a substantial threefold augmentation in the expression of the *MYC* gene in HeLa cells following *EBNA1* transfection **(22)**. Consequently, given MYC's multifaceted proto-oncogenic nature, the heightened expression of MYC induced by *EBNA1* in astrocyte cells infected with EBV could potentially expedite the onset and advancement of GBM.

Additionally, we identified a great elevation in the transcript level of the *BIRC5* gene in U87MG cells transfected with the *EBNA1*-containing plasmid when contrasted with control cells, although this change was not significant. *BIRC5* gene product (also named survivin) is missing in healthy differentiated tissues but is highly expressed in the majority of tumors **(56)**. Survivin controls the progression of the cell cycle, prevents apoptosis, and causes instabilities in chromosomes **(57, 58)**. This protein suppresses cell death by interfering with caspases, which is why its expression has been shown to rise in many cancers **(59)**. Accumulating evidence has revealed that there is a linear relationship between survivin gene transcript level and malignant phenotype in glioma **(60)**. According to Tong *et al.*, the expression of survivin is correlated with a poor prognosis among GBM patients **(56)**. In accordance with our research, a study conducted by Lu and colleagues reported that the viral protein *EBNA1* induces the generation of survivin in Burkitt's lymphoma. This induction occurs through the formation of a complex and its attachment to the survivin promoter **(61)**. Additionally, our previous investigation demonstrated a significant enhancement of *BIRC5* expression by *EBNA1* in a cervical cell line **(22)**.

While our study has successfully identified several differentially expressed mRNAs when comparing *EBNA1*-transfected cells with the control group, it's important to acknowledge the existence of certain limitations. One critical aspect is the necessity to validate these findings at the protein level using alternative techniques, such as western blotting, to enhance the robustness and reliability of the results. This step should be considered in future investigations to overcome this particular limitation. Additionally, the study was constrained by the availability of only the U87MG cell line for our research, which limits the extent of comparative analysis. To address this constraint and enhance the comprehensiveness of our understanding, we recommend the design of further studies involving a broader range of glioblastoma astrocytoma cell lines or even clinical samples. Such studies could encompass an evaluation of various cellular and viral genes at both the mRNA and protein levels. This approach has the potential to yield novel insights into the intricate interplay of EBV in the development of various brain conditions while shedding light on the multifaceted biological roles of *EBNA1* in the context of carcinogenesis. These endeavors would undoubtedly contribute to a more comprehensive understanding of the molecular mechanisms underpinning the influence of EBV on brain health and disease.

5. Conclusion

The findings from our study provide compelling evidence that the presence of *EBNA1* can elevate the expression levels of a range of critical cellular genes, namely *MDMX*, *MDM2*, *MYC*, and *BIRC5*, within U87MG cells. This observation suggests that EBV infection, facilitated by the EBNA1 protein, can exert a substantial influence on the pathophysiology of GBM. This influence extends to the potential exacerbation of the disease's conditions, thereby underscoring the multifaceted role of EBV in the context of GBM development and progression.

Acknowledgment

We sincerely thank all colleagues who helped us in carrying out and advancing this study.

Ethical Considerations

This study was conducted in compliance with ethical guideline (IR.SUMS.REC.1401.003) and does not involve any research with human participants or animals.

Conflict of Interest

No conflicts of interest are disclosed by the authors.

Authors' Contribution

A.H.A., J.S., and A.G.L. designed and administrated the project. A.H.A. wrote the manuscript. S.M.A.H. and H.N. edited the manuscript. A.H.A. and Z.Z.K. performed the experiment. A.H.A. and S.M.A.H. analyzed the data. All authors read and approved the final version of the manuscript.

Funding

This work was not supported by any funding.

References

1. Bleeker FE, Molenaar RJ, Leenstra S. Recent advances in the molecular understanding of glioblastoma. J Neuro-Oncol. 2012;108:11-27. [DOI:10.1007/s11060-011-0793-0] [PMID] [PMCID]
2. Hanif F, Muzaffar K, Perveen K, Malhi SM, Simjee SU. Glioblastoma multiforme: a review of its epidemiology and pathogenesis through clinical presentation and treatment. Asian Pac J Cancer Prev. 2017;18(1):3.
3. de Oliveira DE, Müller-Coan BG, Pagano JS. Viral carcinogenesis beyond malignant transformation: EBV in the progression of human cancers. Trends Microbiol. 2016;24(8):649-64. [DOI:10.1016/j.tim.2016.03.008] [PMID] [PMCID]
4. Kofman A, Marcinkiewicz L, Dupart E, Lyshchev A, Martynov B, Ryndin A, et al. The roles of viruses in brain tumor initiation and oncomodulation. J Neuro-Oncol. 2011;105:451-66. [PMID] [PMCID] [DOI:10.1007/s11060-011-0658-6]
5. Epstein MA, Achong BG, Barr YM. Virus particles in cultured lymphoblasts from Burkitt's lymphoma. The Lancet. 1964;283(7335):702-3. [DOI:10.1016/S0140-6736(64)91524-7] [PMID]
6. Zhang G, Yu Z, Shen G, Chai Y, Liang C. Association between Epstein-Barr virus and Thymic epithelial tumors: a systematic review. Infect. Agents Cancer. 2019;14:1-8. [PMID] [PMCID] [DOI:10.1186/s13027-019-0254-5]
7. Khalil M, Enzinger C, Wallner-Blazek M, Scarpatetti M, Barth A, Horn S, et al. Epstein-Barr virus encephalitis presenting with a tumor-like lesion in an immunosuppressed transplant recipient. J Neurovirol. 2008;14(6):574-8. [DOI:10.1080/13550280802345715] [PMID]
8. Fujimoto H, Asaoka K, Imaizumi T, Ayabe M, Shoji H, Kaji M. Epstein-Barr virus infections of the central nervous system. Intern Med. 2003;42(1): 33-40. [DOI:10.2169/internalmedicine.42.33] [PMID]
9. Menet A, Speth C, Larcher C, Prodinger WM, Schwendinger MG, Chan P, et al. Epstein-Barr virus infection of human astrocyte cell lines. J Virol. 1999;73(9):7722-33. [PMID] [PMCID] [DOI:10.1128/JVI.73.9.7722-7733.1999]
10. Gasque P, Chan P, Mauger C, Schouft MT, Singhrao S, Dierich MP, et al. Identification and characterization of complement C3 receptors on human astrocytes. J Immunol. 1996;156(6):2247-55. [DOI:10.4049/jimmunol.156.6.2247] [PMID]
11. Sugita Y, Muta H, Ohshima K, Morioka M, Tsukamoto Y, Takahashi H, et al. Primary central nervous system lymphomas and related diseases: Pathological characteristics and discussion of the differential diagnosis. Neuropathol. 2016;36(4): 313-24. [DOI:10.1111/neup.12276] [PMID]
12. Jiang L, Xie C, Lung HL, Lo KW, Law GL, Mak NK, et al. EBNA1-targeted inhibitors: Novel approaches for the treatment of Epstein-Barr virus-associated cancers. Theranostics. 2018;8(19):5307-19. [DOI:10.7150/thno.26823] [PMID] [PMCID]
13. Wood VH, O'neil JD, Wei W, Stewart SE, Dawson CW, Young LS. Epstein-Barr virus-encoded EBNA1 regulates cellular gene transcription and modulates the STAT1 and TGFβ signaling pathways. Oncogene. 2007;26(28):4135-47. [DOI:10.1038/sj.onc.1210496] [PMID]
14. Sompallae R, Callegari S, Kamranvar SA, Masucci MG. Transcription profiling of Epstein-Barr virus nuclear antigen (EBNA)-1 expressing cells suggests targeting of chromatin remodeling complexes. PloS One. 2010;5(8):e12052. [PMID] [DOI:10.1371/journal.pone.0012052] [PMCID]
15. Song Q, Liu XQ, Rainey JK. 1H, 15N and 13C backbone resonance assignments of the acidic domain of the human MDMX protein. Biomol NMR Assign. 2022;16(1):171-8. [DOI:10.1007/s12104-022-10081-8] [PMID]
16. Jeyaraj S, O'Brien DM, Chandler DS. MDM2 and MDM4 splicing: an integral part of the cancer spliceome. Front Biosci. 2009;14:2647-56. [DOI:10.2741/3402] [PMID]
17. Dhanasekaran R, Deutzmann A, Mahauad-Fernandez WD, Hansen AS, Gouw AM, Felsher DW. The MYC oncogene-the grand orchestrator of cancer growth and immune evasion. Nat Rev Clin Oncol. 2022;19(1):23-36. [PMID] [PMCID] [DOI:10.1038/s41571-021-00549-2]
18. Li F, Aljahdali I, Ling X. Cancer therapeutics using survivin BIRC5 as a target: what can we do after over two decades of study?. J Exp Clin Cancer Res. 2019;38(1):368. [PMID] [PMCID] [DOI:10.1186/s13046-019-1362-1]
19. Sah NK, Khan Z, Khan GJ, Bisen PS. Structural, functional and therapeutic biology of survivin. Cancer lett. 2006;244(2):164-71. [DOI:10.1016/j.canlet.2006.03.007] [PMID]
20. Hashemi SM, Moradi A, Hosseini SY, Nikoo HR, Bamdad T, Razmkhah M, et al. EBNA1 Upregulates P53-Inhibiting Genes in Burkitt's Lymphoma Cell Line. Rep Biochem Mol Biol. 2023;

11(4):672-83. [DOI:10.52547/rbmb.11.4.672] [PMID] [PMCID]

21. Hashemi SM, Moradi A, Hosseini SY, Nikoo HR, Bamdad T, Faghih Z, et al. A New Insight Into p53-Inhibiting Genes in Epstein-Barr Virus-Associated Gastric Adenocarcinoma. Iran Biomed J. 2023; 27(1):34-45. [DOI:10.52547/ibj.3784] [PMID] [PMCID]
22. Alipour AH, Hashemi SM, Moattari A, Farhadi A, Sarvari J. Epstein-Barr Virus Nuclear Antigen 1 Increases the Expression of HPV Type 18 E6 and E7 Oncogenes and BIRC5/C-MYC Cellular Genes in the Hela Cell Line. Int J Mol and Cell Med. 2022; 11(4):346-56.
23. Schiller JT, Lowy DR. An Introduction to Virus Infections and Human Cancer. In: Wu TC, Chang MH, Jeang KT (eds). Viruses and Human Cancer. Recent Results in Cancer Research, Vol 217. 2021. Springer, Cham. [PMID] [PMCID] [DOI:10.1007/978-3-030-57362-1_1]
24. Ayee R, Ofori ME, Wright E, Quaye O. Epstein Barr virus associated lymphomas and epithelia cancers in humans. J Cancer. 2020;11(7):1737-50. [DOI:10.7150/jca.37282] [PMID] [PMCID]
25. Ghoreshi Z, Molaei H, Arefinia N. The role of DNA viruses in human cancer. Cancer Inform. 2023;22: 11769351231154186. [PMID] [PMCID] [DOI:10.1177/11769351231154186]
26. Ghaffari H, Tavakoli A, Faranoush M, Naderi A, Kiani SJ, Sadeghipour A, et al. Molecular Investigation of Human Cytomegalovirus and Epstein-Barr virus in Glioblastoma Brain Tumor: A Case-Control Study in Iran. Iran Biomes J. 2021; 25(6):426-33. [DOI:10.52547/ibj.25.6.426] [PMID] [PMCID]
27. Fonseca RF, Rosas SL, Oliveira JA, Teixeira A, Alves G, Carvalho MD. Frequency of Epstein-Barr virus DNA sequences in human gliomas. Sao Paulo Med J. 2015;133:51-4. [PMID] [PMCID] [DOI:10.1590/1516-3180.2013.1912814]
28. Frappier L. The Epstein-Barr Virus EBNA1 Protein. Scientifica. 2012;2012:438204. [DOI:10.6064/2012/438204] [PMID] [PMCID]
29. Saridakis V, Sheng Y, Sarkari F, Holowaty MN, Shire K, Nguyen T, et al. Structure of the p53 binding domain of HAUSP/USP7 bound to Epstein-Barr nuclear antigen 1: implications for EBV-mediated immortalization. Mol Cell. 2005; 18(1):25-36. [DOI:10.1016/j.molcel.2005.02.029] [PMID]
30. Boudreault S, Armero VE, Scott MS, Perreault JP, Bisaillon M. The Epstein-Barr virus EBNA1 protein modulates the alternative splicing of cellular genes. Virol J. 2019;16:29. [PMID] [PMCID] [DOI:10.1186/s12985-019-1137-5]
31. Coppotelli G, Mughal N, Callegari S, Sompallae R, Caja L, Luijsterburg MS, et al. The Epstein-Barr virus nuclear antigen-1 reprograms transcription by mimicry of high mobility group A proteins. Nucleic Acids Res. 2013;41(5):2950-62. [DOI:10.1093/nar/gkt032] [PMID] [PMCID]
32. Canaan A, Haviv I, Urban AE, Schulz VP, Hartman S, Zhang Z, et al. EBNA1 regulates cellular gene expression by binding cellular promoters. Proceedings of the National Academy of Sciences. 2009;106(52):22421-6. [DOI:10.1073/pnas.0911676106] [PMID] [PMCID]
33. Toledo F, Wahl GM. MDM2 and MDM4: p53 regulators as targets in anticancer therapy. Int J Biochem Cell Biol. 2007;39(7-8):1476-82. [PMID] [DOI:10.1016/j.biocel.2007.03.022] [PMCID]
34. Swetzig WM, Wang J, Das GM. Estrogen receptor alpha (ERα/ESR1) mediates the p53-independent overexpression of MDM4/MDMX and MDM2 in human breast cancer. Oncotarget. 2016;7(13): 16049-69. [DOI:10.18632/oncotarget.7533] [PMID] [PMCID]
35. Marine JC, Jochemsen AG. MDMX (MDM4), a Promising Target for p53 Reactivation Therapy and Beyond. Cold Spring Harb Perspect Med. 2016;6(7):a026237. [PMID] [PMCID] [DOI:10.1101/cshperspect.a026237]
36. Her NG, Oh JW, Oh YJ, Han S, Cho HJ, Lee Y, et al. Potent effect of the MDM2 inhibitor AMG232 on suppression of glioblastoma stem cells. Cell Death Dis. 2018;9(8):792. [PMID] [PMCID] [DOI:10.1038/s41419-018-0825-1]
37. Dunn GP, Rinne ML, Wykosky J, Genovese G, Quayle SN, Dunn IF, et al. Emerging insights into the molecular and cellular basis of glioblastoma. Genes Dev. 2012;26(8):756-84. [DOI:10.1101/gad.187922.112] [PMID] [PMCID]
38. The Cancer Genome Atlas Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature. 2008;455(7216):1061-8. [DOI:10.1038/nature07385] [PMID] [PMCID]
39. AACR Project Genie Consortium, AACR Project GENIE Consortium, André F, Arnedos M, Baras AS, Baselga J, et al. AACR Project GENIE: Powering Precision Medicine through an International Consortium. Cancer Discov. 2017;7(8):818-31. [DOI:10.1158/2159-8290.CD-17-0151] [PMID] [PMCID]

40. Riemenschneider MJ, Büschges R, Wolter M, Reifenberger J, Boström J, Kraus JA, et al. Amplification and overexpression of the MDM4 (MDMX) gene from 1q32 in a subset of malignant gliomas without TP53 mutation or MDM2 amplification. Cancer Res. 1999;59(24):6091-6.

41. Arjona D, Bello MJ, Alonso ME, Isla A, De Campos JM, Vaquero J, et al. Real-time quantitative PCR analysis of regions involved in gene amplification reveals gene overdose in low-grade astrocytic gliomas. Diagn Mol Pathol. 2005;14(4):224-9. [DOI:10.1097/01.pas.0000177799.58336.1a] [PMID]

42. Spiegelberg D, Mortensen AC, Lundsten S, Brown CJ, Lane DP, Nestor M. The MDM2/MDMX-p53 antagonist PM2 radiosensitizes wild-type p53 tumors. Cancer Res. 2018;78(17):5084-93. [DOI:10.1158/0008-5472.CAN-18-0440] [PMID]

43. Pearson JR, Regad T. Targeting cellular pathways in glioblastoma multiforme. Signal Transduct Target Ther. 2017;2(1):17040. [DOI:10.1038/sigtrans.2017.40] [PMID] [PMCID]

44. Zhang Y, Dube C, Gibert Jr M, Cruickshanks N, Wang B, Coughlan M, et al. The p53 pathway in glioblastoma. Cancers. 2018;10(9):297. [DOI:10.3390/cancers10090297] [PMID] [PMCID]

45. Reifenberger G, Liu L, Ichimura K, Schmidt EE, Collins VP. Amplification and overexpression of the MDM2 gene in a subset of human malignant gliomas without p53 mutations. Cancer Res. 1993;53(12):2736-9.

46. Meyer N, Penn LZ. Reflecting on 25 years with MYC. Nat Rev Cancer. 2008;8(12):976-90. [DOI:10.1038/nrc2231] [PMID]

47. Swartling FJ. Myc proteins in brain tumor development and maintenance. Upsala J Med Sci. 2012;117(2):122-31. [PMID] [PMCID] [DOI:10.3109/03009734.2012.658975]

48. Chattopadhyay P, Banerjee M, Sarkar C, Mathur M, Mohapatra AK, Sinha S. Infrequent alteration of the c-myc gene in human glial tumours associated with increased numbers of c-myc positive cells. Oncogene. 1995;11(12):2711-4.

49. Orian JM, Vasilopoulos K, Yoshida S, Kaye AH, Chow CW, Gonzales MF. Overexpression of multiple oncogenes related to histological grade of astrocytic glioma. Br J Cancer. 1992;66(1):106-12. [DOI:10.1038/bjc.1992.225] [PMID] [PMCID]

50. Zhao K, Wang Q, Wang Y, Huang K, Yang C, Li Y, et al. EGFR/c-myc axis regulates TGFβ/Hippo/Notch pathway via epigenetic silencing miR-524 in gliomas. Cancer Lett. 2017;406:12-21. [DOI:10.1016/j.canlet.2017.07.022] [PMID]

51. Xu Q, Ahmed AK, Zhu Y, Wang K, Lv S, Li Y, et al. Oncogenic MicroRNA-20a is downregulated by the HIF-1α/c-MYC pathway in IDH1 R132H-mutant glioma. Biochem Biophys Res Commun. 2018;499(4):882-8. [DOI:10.1016/j.bbrc.2018.04.011] [PMID]

52. Luo H, Chen Z, Wang S, Zhang R, Qiu W, Zhao L, et al. c-Myc-miR-29c-REV3L signalling pathway drives the acquisition of temozolomide resistance in glioblastoma. Brain. 2015;138(12):3654-72. [DOI:10.1093/brain/awv287] [PMID]

53. Zhang G, Zhu Q, Fu G, Hou J, Hu X, Cao J, et al. TRIP13 promotes the cell proliferation, migration and invasion of glioblastoma through the FBXW7/c-MYC axis. Br J Cancer. 2019;121(12): 1069-78. [DOI:10.1038/s41416-019-0633-0] [PMID] [PMCID]

54. Ding Z, Liu X, Liu Y, Zhang J, Huang X, Yang X, et al. Expression of far upstream element (FUSE) binding protein 1 in human glioma is correlated with c-Myc and cell proliferation. Mol Carcinog. 2015;54(5):405-15. [DOI:10.1002/mc.22114] [PMID]

55. Wang J, Wang H, Li Z, Wu Q, Lathia JD, McLendon RE, et al. c-Myc is required for maintenance of glioma cancer stem cells. PloS One. 2008;3(11): e3769. [DOI:10.1371/journal.pone.0003769] [PMID] [PMCID]

56. Jaskoll T, Chen H, Min Zhou Y, Wu D, Melnick M. Developmental expression of survivin during embryonic submandibular salivary gland development. BMC Dev Biol. 2001;1:5. [DOI:10.1186/1471-213X-1-5] [PMID] [PMCID]

57. Conde M, Michen S, Wiedemuth R, Klink B, Schröck E, Schackert G, et al. Chromosomal instability induced by increased BIRC5/Survivin levels affects tumorigenicity of glioma cells. BMC Cancer. 2017;17:889. [PMID] [PMCID] [DOI:10.1186/s12885-017-3932-y]

58. Sheng L, Wan B, Feng P, Sun J, Rigo F, Bennett CF, et al. Downregulation of Survivin contributes to cell-cycle arrest during postnatal cardiac development in a severe spinal muscular atrophy mouse model. Hum Mol Genet. 2018;27(3):486-98. [DOI:10.1093/hmg/ddx418] [PMID] [PMCID]

59. Ye HB, Ma BJ, Meng GQ, Tao S, Wang Y, Chen Z, et al. Bioinformatics analysis of BIRC5 in human cancers. Ann Transl Med. 2022;10(16):888. [DOI:10.21037/atm-22-3496] [PMID] [PMCID]

60. Li PL, Zhang X, Wang LL, Du LT, Yang YM, Li J, et al. MicroRNA-218 is a prognostic indicator in colorectal cancer and enhances 5-fluorouracil-induced apoptosis by targeting BIRC5. Carcinogenesis. 2015;36(12):1484-93. [DOI:10.1093/carcin/bgv145] [PMID]

61. Lu J, Murakami M, Verma SC, Cai Q, Haldar S, Kaul R, et al. Epstein-Barr Virus nuclear antigen 1 (EBNA1) confers resistance to apoptosis in EBV-positive B-lymphoma cells through up-regulation of survivin. Virology. 2011;410(1):64-75. [PMID] [DOI:10.1016/j.virol.2010.10.029] [PMCID]