Research Article

Curcumin Inhibits Proliferation of Epstein–Barr Virus-Associated Human Nasopharyngeal Carcinoma Cells by Inhibiting EBV Nuclear Antigen 1 Expression

Limei Liu,1 Jiaomin Yang,2 Wuguang Ji,3 and Chao Wang4

1Department of Keratonosus, Weifang Eye Hospital, Weifang 261053, Shandong, China
2Department of Laboratory Medicine, Weifang Yidu Central Hospital, Qingzhou 262500, Shandong, China
3Department of Vascular Surgery, Weifang People’s Hospital, Weifang 261053, Shandong, China
4Department of Pathogen Biology, Weifang Medical University, Weifang 261053, Shandong, China

Correspondence should be addressed to Chao Wang; wangchao96988@126.com

Received 6 May 2019; Revised 31 August 2019; Accepted 12 September 2019; Published 7 October 2019

1.Introduction

Epstein–Barr virus (EBV), a well-known common γ-herpes virus, induces asymptomatic infection in approximately 95% adults throughout the world [1]. The EBV infection has been regarded as the high-risk factor related to more than 1% cancer cases [2]. Moreover, the infection of EBV has also been implied in various proliferative diseases associated with epithelial or lymphoid cells in human beings, such as the gastric carcinoma, nasopharyngeal carcinoma (NPC), Burkitt’s lymphoma, and Hodgkin’s lymphoma [3]. As a malignant tumor, NPC mainly originates from the nasopharynx epithelium, which is endemic in South China and Southeast Asia, and the disease incidence is 20–50 cases per 100,000 people [4, 5]. In NPC, EBV infection has type II latency mechanism [6], which is featured by several noncoding RNAs, latent membrane proteins (LMP1, LMP2A, and LMP2B), and Epstein–Barr nuclear antigen 1 (EBNA1) [7]. Due to the special viral antigen expression, NPC has become an attractive target for clinical therapies.

The essential viral protein EBNA1 is expressed in tumors associated with EBV, during the viral latency processes (but not for latency 0) [8]. EBNA1 plays vital roles in the maintenance, replication, and segregation of latent episomes [9]. Moreover, EBNA1 could interact with the cellular and viral promoters, regulating the gene transcription level [9]. A recent study has shown that EBNA1 has oncogenic activity, and this pleiotropic protein regulates its own synthesis to escape from the host cells exerting immune functions [10]. Furthermore, EBNA1 has also been shown to be able to
decrease p53 accumulation in the epithelial cells infected with EBV, which might disrupt the antitumor function of these cells [11]. Although expressed in malignancies associated with EBV, the EBNA1 expression would obey various regulating modes, which depends on the types of host cells. Under most circumstances, the EBNA1 transcription would be promoted by the Q promoter (Qp) (in both type I or II latency) [12]. For the NPC cells (type II infection), Qp could mediate EBNA1 transcription [13]. Furthermore, it has been suggested that EBNA1 would be necessary the tumor cell proliferation [14]. EBNA1 is recognized as an attractive target in various therapeutic strategies in clinics.

Curcumin (Cur) is a polyphenol extracted from Curcuma longa, which has been extensively investigated due to its modulating effects of signaling pathways related to proliferation and invasion of cells, as well as angiogenesis [15, 16]. Curcumin can exert pharmacological functions, such as antimicrobial and anti-inflammation functions [17–19]. Moreover, curcumin is able to block the cancer growth of various types, including prostate cancer, NPC, and other cancers [20, 21]. Curcumin could reduce human breast cancer cell (MDA-MB-231) proliferation by decreasing the expression level of Bcl-2 and upregulating the Bax, PARP, and cleaved caspase 3 expression levels [22]. A previous study has shown that curcumin could sensitize the NPC cells to radiation via modulating the noncoding RNA levels, Jab1/CSN5, and/or ROS generation [23]. Furthermore, curcumin is shown to cause NPC cell apoptosis through both the mitochondrial-dependent and death receptor-mediated pathways [24].

Our previous study had shown that heat shock protein 90 (Hsp90) inhibitors could block the proliferation of malignant cells infected with EBV, both in vivo and in vitro [25]. It has also been suggested that triptolide treatment could inhibit EBV+ B lymphocyte proliferation through inhibiting LMP1 [26]. Furthermore, we have also shown that berberine shows the antitumor activity against NPC through the EBNA 1-dependent mechanism [13]. Furthermore, triptolide could decrease the human telomerase reverse transcriptase stability in BC-3 and BCBL-1 cells [27].

In this study, effects of curcumin on cell proliferation, cellular apoptosis, and cycle arrest of EBV+ NPC cells were investigated. Moreover, mechanisms through which curcumin inhibited NPC were also analyzed.

2. Materials and Methods

2.1. Study Cells. EBV+ NPC cells (i.e., the HONE1 and HK1-EBV cells) were kind gifts from Prof. SaiWahTsao (University of Hong Kong, Hong Kong, China). These cells were cultured with the RPMI-1640 medium, which contained G418 (400 ng/ml) and 10% fetal bovine serum (Gibco-BRL). HeLa cells were cultured in the DMEM medium, which also contained 10% fetal bovine serum. These cells were cultured at 37°C with 5% CO₂.

2.2. Cellular Viability Assessment. Cellular viability was analyzed with the Cell Counting Kit-8 (CCK-8) (Dojindo Laboratories). The cells were planted onto the 96-well plate (1 x 10⁴ cells/100μl), which were incubated with DMEM. These cells were incubated with 0.006% DMSO (vehicle control) or increasing concentrations of curcumin (Sigma-Aldrich, Shanghai, China), for 24 and 48h, respectively. Then, 10μl CCK-8 was added to incubate the cells in dark at 37°C for 1 h. The OD₄₅₀ value was detected with an EL x 800 microimmuno analyzer (BioTek Instruments, Inc.).

2.3. Flow Cytometry. Annexin V-FITC/PI apoptosis detection kit (Multisciences) was used to analyze apoptosis. HK1-EBV and HONE1 cells were incubated with 0.006% DMSO (vehicle control) or curcumin (5μM and 10μM). After 24h, cells were resuspended in the binding buffer (500μl). The cells were incubated with Annexin V-FITC (5μl) and PI (10μl). After that, cells were analyzed with EPICS Altra II (Beckman Coulter).

2.4. Transfection. The plasmids of pSG-5-EBNA1 and pGL3.0-Qp were synthesized by the Neuron Biotech Corporation (Shanghai, China). Transient cellular transfection was performed with X-tremeGENE HP DNA Transfection Reagent (Roche). After 4h of transfection, 0.006% DMSO (control) or curcumin was added and incubated for 44h.

2.5. Dual Luciferase Reporter Assay. After cotransfection with 100 ng pGL3.0-Qp and 5 ng pRL-TK for 4h, HeLa cells were incubated with 0.006% DMSO or curcumin (5 or 10μM). After 24h, the total protein was harvested with the 1X lysis buffer (Promega, Madison, WI, USA). Luciferase activity was measured by the GLO-MAX 20/20 system (Promega, Madison, WI, USA). Renilla was as internal control reference.

2.6. Quantitative Real-Time PCR. The total RNA was extracted with TRIzol (Invitrogen, Grand Island, NY, USA). First-strand cDNA was synthesized with the Reverse Transcription kit (Takara, Tokyo, Japan). Quantitative real-time PCR was conducted on the CFX96 real-time PCR detection system, with the SYBR Premix Ex Taq kit (Takara, Tokyo, Japan). Primer sequences were as follows: EBNA1, forward 5’-GGTCGTGGACGTGGAGAAAA-3’ and reverse 5’-GGTGAGAACCGGATGAGT-3’; and GAPDH, forward 5’-ACATCGTCAGACACCATG-3’ and reverse 5’-TGTAGTGGTACATGAAGGG-3’. The reaction conditions were set as follows: 95°C for 30s; 95°C for 10 s; 62°C for 10 s, and 72°C for 15 s, for a total of 45 cycles. The house-keeping gene GAPDH was as internal reference.

2.7. Western Blotting. Cells were lysed with the RIPA buffer (Beyotime, Shanghai, China), containing 0.5% cocktail protease inhibitor (Roche) and 0.5 mM PMS, on a microscrap. After sonication for 15 s, the extract was centrifuged (12,000 x g) for 15 min. Protein concentration was
3. Results

3.1. Curcumin Suppresses Proliferation of NPC Cells Positive for EBV. To investigate the effects of curcumin treatment on the cellular viability of NPC cells positive for EBV, HONE1, and HK1-EBV, cell proliferation was assessed with the CCK-8, after drug administration. As shown in Figure 1, curcumin reduced HONE1 cell viability, in not only a dose-dependent manner but also a time-dependent manner. Moreover, the decline in HONE1 cell viability ranged over 35%–90% after treatment with curcumin for 24 h and 48 h. For the HONE1 cells, the 50% inhibitory concentration (IC50) values were 12.4 μM and 3.3 μM for the 24 h and 48 h curcumin treatment, respectively. On the contrary, similar findings were obtained for the HK1-EBV cells subjected to the treatment series of curcumin. Moreover, after 24 h and 48 h curcumin treatment, the HK1-EBV cell viability was reduced from 22% to 81%. Furthermore, for the HK1-EBV cells, the IC50 values were 10.4 μM and 7.2 μM for the 24 h and 48 h curcumin treatment, respectively (Figure 1(c)). In addition, the concentration series of curcumin treatment slightly inhibited the proliferation of the HUVE and HK2 cells (Figures 1(d) and 1(e)). These findings demonstrate that curcumin could efficiently decrease the EBV+ NPC cell viabilities but not for the HUVE and HK2 cells.

3.2. Curcumin Leads to Cell Cycle Arrest in NPC Cells Positive for EBV. To investigate the effects of curcumin on the cell cycle of NPC cells positive for EBV, flow cytometry was performed after drug treatment. The treatment of 10 μM curcumin led to an arrest in the S phase in the HONE1 cells, with the fraction of S-phased cells increased by 25.6% (Figure 2). On the contrary, in the HK1-EBV cells, the curcumin treatment (10 μM) led to a G2 arrest, with the cell fraction of G2-phase increased by 15.7%. These results reveal that curcumin could induce cell cycle arrest of NPC cells positive for EBV.

3.3. Curcumin Enhances NPC Cell Apoptosis via Mitochondria- and Death Receptor-Dependent Pathways. Effect of curcumin treatment on NPC cell apoptosis was analyzed. As shown in Figure 3, the curcumin treatment enhanced apoptosis in the HK1-EBV and HONE1 cells, in a dose-dependent manner. Moreover, the apoptosis-related protein expression levels had been examined with the western blotting. Curcumin decreased the caspase-9 level, while increased the cleaved caspase-3, p53, cleaved PARP-1, Fas, and FasL expression levels, in the HK1-EBV and HONE1 cells (Figure 3). The findings suggest that curcumin could induce NPC cell apoptosis via both the mitochondria- and death receptor-dependent pathways.

3.4. Curcumin Downregulated EBNA1 Expression Levels in NPC Cells Positive for EBV. To investigate whether curcumin could alter the expression levels of EBNA1 in the NPC cells positive for EBV after drug administration, the HK1-EBV and HONE1 cells were detected with western blotting. EBNA1 levels in the HONE1 cells were not dramatically altered in the 5 μM curcumin treatment group compared with the control group, which were downregulated to 29.8% for the 10 μM curcumin treatment group (Figures 4(a) and 4(b)). Moreover, the EBNA1 expression levels in the HK1-EBV cells were decreased to 30.5% and 12.7% of the control group, after curcumin treatment (5 and 10 μM). At the same time, another important protein expressed by EBV, LMP1, was also investigated. Our results showed that curcumin could not affect the expression of LMP1 in HONE1 and HK1-EBV cells.

To determine if curcumin specifically decreases the EBNA1 expression levels, HeLa cells were transfected with pSG5-EBNA1 for 4 h, which were then subjected to treatment with or without curcumin. In the pSG5-EBNA1-transfected HeLa cells, the EBNA1 level was decreased to 42.5% and 17.6% for the treatments of curcumin at 5 μM and 10 μM, respectively (Figure 4(c)). These results were consistent with the results obtained from the cell apoptosis assay.

To further analyze whether the decreased EBNA1 expression level was induced by the alterations of the transcription level, quantitative real-time PCR was carried out for the HK1-EBV and HONE1 cells after drug administration. Our findings showed that compared with the control group, curcumin (5 μM and 10 μM) reduced the EBNA1 mRNA levels in the HONE1 cells to 70.12% and 39.52%, respectively. On the contrary, EBNA1 mRNA levels in HK1-EBV cells had been reduced to 60.18% and 37.26% for the 5 μM and 10 μM treatment groups, respectively (Figure 4(d)). The findings suggest that curcumin could inhibit the EBNA1 levels in the HK1-EBV and HONE1 cells.
3.5. Curcumin Decreases EBNA1 Promotor Qp Activities. EBNA1 transcription is initiated by Qp in EBV+ NPC cells. To determine the effects of curcumin on the EBNA1 promotor activity, the HeLa cells were transfected with pRL-TK, in combination with pGL3.0 or pGL3.0-Qp vector. After drug administration, the luciferase activity was measured. The Qp activities were reduced to 70.2% and 59.8% for the 5 μM and 10 μM curcumin treatment groups, respectively, (Figure 4(e)). Thus, curcumin treatment could decrease the transcriptional activity of EBNA1 in the HeLa cells.

3.6. Curcumin Reduces Stability and Promotes Proteasomal Degradation of EBNA1. To determine the effects of curcumin on the stability of EBNA1, the HONE1 and HK1-EBV cells were subjected to curcumin, under the condition of cycloheximide (CHX). Curcumin reduced the half-time of EBNA1 in HK1-EBV and HONE1 cells after CHX treatment compared with the control groups (Figures 4(f) and 4(g)). In addition, the effects of curcumin on the proteasome pathway were investigated. HK1-EBV and HONE1 cells were incubated with curcumin, with or without the proteasome inhibitor (MG-132). MG-132 treatment
Figure 2: Curcumin induces cell cycle arrest in EBV-positive NPC cells. (a) Effect of curcumin on the cell cycle. HONE1 and HK1-EBV cells were treated with DMSO (vehicle control) or curcumin at indicated concentrations, for 24 h. Cell cycle was analyzed by flow cytometry. (b) Statistical analysis of the cell cycle. Compared with the vehicle control (0.006% DMSO) group, *P < 0.05, **P < 0.01.

Figure 3: Curcumin induces cell apoptosis in EBV-positive NPC cells. (a) Effect of curcumin on cell apoptosis. HONE1 and HK1-EBV cells were treated with DMSO (vehicle control) or curcumin at indicated concentrations, for 24 h. Cellular apoptosis was analyzed by flow cytometry. (b) HONE1 and HK1-EBV cells were treated with DMSO (vehicle control) or curcumin at indicated concentrations, for 48 h, and the related protein expression levels were determined by western blot analysis.
Figure 4: Effects of curcumin on transcription, expression, and half-time of EBNA1 in HONE1 and HK1-EBV cells. (a) HONE1 and HK1-EBV cells treated with DMSO (vehicle control) or curcumin (5 μM and 10 μM), for 48 h. (b) Statistical analysis of the expression levels of EBNA1 in the HONE1 and HK1-EBV cells. (c) HeLa cells transfected with pSG5 or pSG5-EBNA1, followed by the treatment of curcumin (5 μM and 10 μM) for 44 h. (d) HONE1 and HK1-EBV cells treated with DMSO (vehicle control) or curcumin (5 μM and 10 μM), for 24 h. (e) HeLa cells cotransfected with pRL-TK and pGL3.0-Qp, followed by the treatment with DMSO (vehicle control) or curcumin (5 μM and 10 μM). The activity of the promoter was determined by the Dual-Luciferase reporter assay. (f) HONE1 (f) and HK1-EBV (g) cells treated with DMSO (vehicle control) or curcumin (10 μM), in the presence or absence of CHX (50 μg/ml). (h) HONE1 (h) and HK1-EBV (i) cells treated with DMSO (vehicle control) or curcumin (10 μM), in the presence or absence of MG-132 (50 μg/ml). Transcription levels of EBNA1 were determined by quantitative real-time PCR. Western blot analysis was performed to detect the protein expression levels. Compared with the vehicle control (0.006% DMSO) group, *P < 0.05, **P < 0.01.
increased the EBNA1 expression levels (Figures 4(h) and 4(i)) in both the HK1-EBV and HONE1 cells, either with or without curcumin. These findings suggest that curcumin could induce the EBNA1 degradation via the proteasome-ubiquitin pathway.

3.7. EBNA1 Overexpression Attenuates Effects of Curcumin. Whether the curcumin-decreased cell viability was induced by the inhibited EBNA1 expression was determined. The HK1 and HONE1 cells were transfected with pSG5-EBNA1 and then treated with curcumin. The transient transfection increased the EBNA1 expression levels in the HONE1 cells by 301.3% and in the HK1 cells by 368.1% (Figures 5(a) and 5(b)). The cellular viability was then determined. As shown in Figures 5(c) and 5(d), curcumin treatment significantly decreased the cell viabilities of both HK1-EBV and HONE1 cells. After EBNA1 overexpression for 48 h, the cell viabilities of HONE1 and HK1-EBV cells were increased by 15.8% and 10.4%, respectively. The findings demonstrate that curcumin could inhibit EBV+ HONE1 and HK1-EBV cell proliferation, probably via decreasing EBNA1 expression.

3.8. Curcumin Inhibits Replication of EBV in NPC Cells Positive for EBV. Effect of curcumin on the replication of EBV was determined. The TPA and NaB-induced HK1-EBV and HONE1 cells were treated with curcumin. Quantitative real-time PCR was performed to investigate the latent and lytic replication of EBV EBNA1 fragment. Curcumin treatment reduced the production of progeny virion, in a dose-dependent manner (Figures 6(a) and 6(b)). The findings demonstrate that curcumin could inhibit EBV latent and lytic replication in both HK1-EBV and HONE1 cells. Moreover, the effect of Curcumin on the EBV transcriptional activator BZLF1 encoded by IE was investigated with western blot analysis. Our results showed that curcumin significantly reduced the expression levels of BZLF1 in both induced and noninduced HONE1 and HK1-EBV cells.

4. Discussion

It has been demonstrated that the infection of EBV is closely linked to NPC [28]. EBNA1 could regulate the DNA synthesis of EBV, which contributes to the cellular proliferation, survival, tumorigenesis, and immortalization, which provides an attractive target for the EBV-related diseases [4, 29, 30]. Previous studies from our lab have shown that the Hsp90 inhibitor and berberine could inhibit the proliferation of malignant cells infected with EBV via the EBNA1-dependent pathway [13, 25]. It is clinically significant to find and develop the drugs that could inhibit the activity of EBNA1. Herein, our findings indicated that curcumin reduced the EBNA1 expression via the proteasome-ubiquitin pathway, both in vivo and in vitro. Moreover, EBNA1 overexpression could attenuate the curcumin-induced NPC cell apoptosis.

Curcumin is a rhizome extracted from the herb *Curcuma longa* Linn., which is frequently used in Ayurveda and traditional Chinese medicine to prevent and cure a variety of ailments in human beings [31, 32]. It has been shown that curcumin might be applied as efficient treatments for various disorders, including the allergy, coryza, sinusitis, hepatic diseases, cough, asthma, and bronchial hyperactivity [33–35]. Moreover, curcumin could exert the antioxidant, antiarthritic, hepatoprotective, thrombosuppressive, and cardioprotective effects [36, 37]. Furthermore, due to its distinct chemical properties, curcumin has also been reported as a potent anticancer compound [38, 39]. Curcumin could interact with various intracellular and extracellular molecules actively implied in the initiation as well as progression of cancers, thereby inhibiting the disease progression [40].

Increasing evidence has suggested that the inflammatory pathways play pivotal roles in a variety of chronic diseases, including cancers [41]. EBNA1 is a vital protein for the replication and persistence of EBV episomes [12, 42]. Herein, curcumin reduced the EBNA1 level in the EBV+ NPC cells and EBV+ HeLa cells, which had pSG5-EBNA1 transfection. In addition, the EBNA1 stability was declined by curcumin treatment. Curcumin could inhibit the mRNA and protein levels of EBNA1 in the HK1 and HONE1 cells.

A previous study from our lab has indicated that triptolide could suppress the LMP1 promoter ED-L1 activity in the B lymphocytes positive for latency III type infected EBV [26]. In NPC, the EBV would express type II latency. Herein, the EBNA1 mRNA expression levels were decreased by curcumin treatment. Moreover, the EBNA1 promoter Qp activity was inhibited by the curcumin treatment, indicating that the decreased activity of the EBNA1 promoter would inhibit the transcription levels of EBNA1.

Previous studies have suggested that curcumin could activate caspase-3, caspase-8, and caspase-9 and PARP protein in various cancer cell lines [43, 44]. However, increasing evidence has demonstrated that the essential apoptotic pathways represent the mitochondria-dependent and death receptor-mediated pathways [45]. In this study, our results suggested that curcumin could induce EBV+ NPC cell apoptosis, through the caspase-9-involved and death receptor-mediated pathways.

In addition to the EBV-encoded protein latent membrane protein 1 (LMP1), EBNA1 also regulates the viral transcription and exerts multiple functions on cellular proteins, as well as on the pathways promoting cell survival and proliferation [14]. A previous study from our lab has shown that the LMP1 overexpression would increase the EBV+ B lymphocyte (B95-8 and P3HR) viabilities [26]. Therefore, we speculate whether the EBNA1 overexpression could increase the viabilities of HK1-EBV and HONE1 cells. Our findings showed that EBNA1 overexpression attenuated the inhibitory effects of curcumin. Moreover, the curcumin treatment reduced the EBV+ NPC cell proliferation and activities, at least partially, related to the reduced EBNA1 level.
Figure 6: Curcumin reduces EBV virion production in EBV-positive NPC cells. The HONE1 (a) and HK1-EBV (b) cells were treated with TPA (40 ng/ml) and SB (3 mM) for 3 h followed by the treatment of DMSO (vehicle control) or curcumin (5 μM and 10 μM). The culture medium was collected and subjected to quantitative real-time PCR. Compared with the vehicle control (0.006% DMSO) group, *P < 0.05, **P < 0.01.
5. Conclusions

In conclusion, our findings indicate that curcumin reduced cell proliferation and apoptosis and changed cycle arrest in the EBV+ NPC cells. Moreover, the decreased Qp promoter activity provides evidence for curcumin-induced declined EBNA1 mRNA levels. In addition, EBNA1 overexpression attenuates the effects of curcumin in EBV+ NPC cells. Based on these findings, curcumin might make a novel therapy for a variety of tumors in clinics, including NPC. Finally, although our results suggested that curcumin might reduce the total number of EBV-positive cells in vitro, the in vivo test should be carried out, and the long-term toxicity of curcumin should be estimated. Moreover, the safety and efficacy of curcumin would need to be investigated in clinical trials.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

This work was supported by the Science and Technology Bureau of Weifang Kuiven District (No. 2018-3).

References

[1] L. Fitzsimmons and G. L. Kelly, “EBV and apoptosis: the viral master regulator of cell fate?,” Viruses, vol. 9, no. 11, 2017.
[2] Y.-J. Xu, R. Zhou, J.-F. Zong et al., “Epstein-Barr virus-coded miR-BART313 promotes nasopharyngeal carcinoma cell growth and metastasis via targeting of the NKIRAS2/NF-κB pathway,” Cancer Letters, vol. 447, pp. 33–40, 2019.
[3] D. Elgui de Oliveira, B. G. Müller-Coan, and J. S. Pagano, “Viral carcinogenesis beyond malignant transformation: EBV in the progression of human cancers,” Trends in Microbiology, vol. 24, no. 8, pp. 649–664, 2016.
[4] J. Dheekollu, K. Malecka, A. Wiedmer et al., “Carcinoma-risk variant of EBNA1 deregulates Epstein-Barr virus epismal latency,” Oncotarget, vol. 8, no. 5, pp. 7248–7264, 2017.
[5] Y. Nakanishi, N. Wakisaka, S. Kondo et al., “Progression of understanding for the role of Epstein-Barr virus and management of nasopharyngeal carcinoma,” Cancer and Metastasis Reviews, vol. 36, no. 3, pp. 435–447, 2017.
[6] J. Zhang, L. Jia, C. M. Tsang, and S. W. Tsao, “EBV infection and glucose metabolism in nasopharyngeal carcinoma,” Advances in Experimental Medicine and Biology, vol. 1018, pp. 75–90, 2017.
[7] A. Nanbo, H. Katano, M. Kataoka et al., “Infection of Epstein-Barr virus in type III latency modulates biogenesis of exosomes and the expression profile of exosomal miRNAs in the Burkitt lymphoma mutu cell lines,” Cancers, vol. 10, no. 7, p. 237, 2018.
[8] L. Jiang, C. Xie, H. L. Lung et al., “EBNA1-targeted inhibitors: novel approaches for the treatment of Epstein-Barr virus-associated cancers,” Therapeutics, vol. 8, no. 19, pp. 5307–5319, 2018.
[9] J. S. Deakyne, K. A. Malecka, T. E. Messick, and P. M. Lieberman, “Structural and functional basis for an EBNA1 hexameric ring in Epstein-Barr virus episome maintenance,” Journal of Virology, vol. 91, no. 19, 2017.
[10] M. Albanese, T. Tagawa, M. Bouwet et al., “Epstein-Barr virus microRNAs reduce immune surveillance by virus-specific CD8+ T cells,” Proceedings of the National Academy of Sciences, vol. 113, no. 42, pp. E6467–E6475, 2016.
[11] S. AiQarni, Y. Al-Sheikh, D. Campbell et al., “Lymphomas driven by Epstein-Barr virus nuclear antigen-1 (EBNA1) are dependent upon Mdm2,” Oncogene, vol. 37, no. 29, pp. 3998–4012, 2018.
[12] R. J. A. Verhoeven, S. Tong, J. Zong et al., “NF-κBappa signaling regulates Epstein-Barr virus BambHi-Q-driven EBNA1 expression,” Cancers, vol. 10, no. 4, p. 119, 2018.
[13] C. Wang, H. Wang, Y. Zhang et al., “Berberine inhibits the proliferation of human nasopharyngeal carcinoma cells via an Epstein-Barr virus nuclear antigen I-dependent mechanism,” Oncology Reports, vol. 37, no. 4, pp. 2109–2120, 2017.
[14] Y. Zhang, H. Wang, Y. Liu et al., “Baicalin inhibits growth of Epstein-Barr virus-positive nasopharyngeal carcinoma by repressing the activity of EBNA1 Q-promoter,” Biomedicine & Pharmacotherapy, vol. 102, pp. 1003–1014, 2018.
[15] S. Mapoung, S. Suzuki, S. Fuji et al., “Cyclohexanone curcumin analogs inhibit the progression of castration-resistant prostate cancer in vitro and in vivo,” Cancer Science, vol. 110, no. 2, pp. 596–607, 2019.
[16] T. Tsuda, “Curcumin as a functional food-derived factor: degradation products, metabolites, bioactivity, and future perspectives,” Food & Function, vol. 9, no. 2, pp. 705–714, 2018.
[17] K.-Y. Chin, “The spice for joint inflammation: anti-inflammatory role of curcumin in treating osteoarthritis,” Drug Design, Development and Therapy, vol. 10, pp. 3029–3042, 2016.
[18] P. V. Sanita, A. C. Pavarina, L. N. Dovigo, A. P. D. Ribeiro, M. C. Andrade, and E. G. D. O. Mima, “Curcumin-mediated anti-microbial photodynamic therapy against Candida dublieniensis biofilms,” Lasers in Medical Science, vol. 33, no. 4, pp. 709–717, 2018.
[19] Y. Yao, W. Wang, M. Li et al., “Curcumin exerts its anti-hypertensive effect by down-regulating the AT1 receptor in vascular smooth muscle cells,” Scientific Reports, vol. 6, no. 1, p. 25579, 2016.
[20] J. Adiwidjaja, A. J. McLachlan, and A. V. Boddy, “Curcumin as a clinically-promising anti-cancer agent: pharmacokinetics and drug interactions,” Expert Opinion on Drug Metabolism & Toxicology, vol. 13, no. 9, pp. 953–972, 2017.
[21] K. Yoshida, S. Toden, P. Ravindranathan, H. Han, and A. Goel, “Curcumin sensitizes pancreatic cancer cells to gemcitabine by attenuating PRC2 subunit EZH2, and the IncRNA PVT1 expression,” Carcinogenesis, vol. 38, no. 10, pp. 1036–1046, 2017.
[22] S. Hu, Y. Xu, L. Meng, L. Huang, and H. Sun, “Curcumin inhibits proliferation and promotes apoptosis of breast cancer cells,” Experimental and Therapeutic Medicine, vol. 16, no. 2, pp. 1266–1272, 2018.
[23] A. A. Momtazi-Borjoeini, F. Ghasemi, A. Hesari, M. Majeed, M. Caraglia, and A. Sahebkar, “Anti-cancer and radio-sensitizing effects of curcumin in nasopharyngeal carcinoma,” Current Pharmaceutical Design, vol. 24, no. 19, pp. 2121–2128, 2018.
[24] F. W. Yang, J. Z. Huang, X. L. Lin, Z. N. Zhen, and X. M. Chen, “Apoptosis in nasopharyngeal carcinoma cell line NCE induced by curcumin and its molecular mechanism,” Zhonghua Er Bi Yan Hou Tou Jing Wai Ke Za Zhi, vol. 41, no. 8, pp. 612–616, 2006.

[25] X. Sun, E. A. Barlow, S. Ma et al., “Hsp90 inhibitors block outgrowth of EBV-infected malignant cells in vitro and in vivo through an EBNA1-dependent mechanism,” Proceedings of the National Academy of Sciences, vol. 107, no. 7, pp. 3146–3151, 2010.

[26] H. Zhou, W. Guo, C. Long, H. Wang, J. Wang, and X. Sun, “Triptolide inhibits proliferation of Epstein-Barr virus-positive B lymphocytes by down-regulating expression of a viral protein LMP1,” Biochemical and Biophysical Research Communications, vol. 456, no. 3, pp. 815–820, 2015.

[27] C. Long, J. Wang, W. Guo et al., “Triptolide inhibits transcription of hTERT through down-regulation of transcription factor specificity protein 1 in primary effusion lymphoma cells,” Biochemical and Biophysical Research Communications, vol. 469, no. 1, pp. 87–93, 2016.

[28] S. W. Tsao, C. M. Tsang, and K. W. Lo, “Epstein-Barr virus infection and nasopharyngeal carcinoma,” Philosophical Transactions of the Royal Society B: Biological Sciences, vol. 372, no. 1732, 2017.

[29] L. Frappier, “Contributions of Epstein-Barr nuclear antigen 1 (EBNA1) to cell immortalization and survival,” Viruses, vol. 4, no. 9, pp. 1537–1547, 2012.

[30] I. Tempera, A. De Leo, A. V. Kossenkov et al., “Identification of MEF2B, EBF1, and IL6R as direct gene targets of Epstein-barr virus (EBV) nuclear antigen 1 critical for EBV-infected B-lymphocyte survival,” Journal of Virology, vol. 90, no. 1, pp. 345–355, 2016.

[31] T. Arablou and R. Kolahdouz-Mohammadi, “Curcumin and endometriosis: review on potential roles and molecular mechanisms,” Biomedicine & Pharmacotherapy, vol. 97, pp. 91–97, 2018.

[32] P. P. Desai and V. B. Patrawale, “Curcumin cocystal micelles-multifunctional nanocomposites for management of neurodegenerative ailments,” Journal of Pharmaceutical Sciences, vol. 107, no. 4, pp. 1143–1156, 2018.

[33] L. Birdane, C. Cingi, N. B. Muluk, T. San, and D. Burukoglu, “Evaluation of the efficacy of curcumin in experimentally induced acute sinusitis in rats,” Ear, Nose & Throat Journal, vol. 95, no. 12, pp. E21–E27, 2016.

[34] J. Liang, X. Dong, A. Yang, D. Zhu, D. Kong, and F. Lv, “A dual fluorescent reverse targeting drug delivery system based on curcumin-loaded ovalbumin nanoparticles for allergy treatment,” Nanomedicine: Nanotechnology, Biology and Medicine, vol. 16, pp. 56–68, 2019.

[35] T. Arablou and R. Kolahdouz-Mohammadi, “Curcumin and endometriosis: review on potential roles and molecular mechanisms,” Biomedicine & Pharmacotherapy, vol. 97, pp. 91–97, 2018.

[36] P. P. Desai and V. B. Patrawale, “Curcumin cocystal micelles-multifunctional nanocomposites for management of neurodegenerative ailments,” Journal of Pharmaceutical Sciences, vol. 107, no. 4, pp. 1143–1156, 2018.

[37] L. Birdane, C. Cingi, N. B. Muluk, T. San, and D. Burukoglu, “Evaluation of the efficacy of curcumin in experimentally induced acute sinusitis in rats,” Ear, Nose & Throat Journal, vol. 95, no. 12, pp. E21–E27, 2016.

[38] J. Liang, X. Dong, A. Yang, D. Zhu, D. Kong, and F. Lv, “A dual fluorescent reverse targeting drug delivery system based on curcumin-loaded ovalbumin nanoparticles for allergy treatment,” Nanomedicine: Nanotechnology, Biology and Medicine, vol. 16, pp. 56–68, 2019.

[39] M. Namdari and A. Eatemadi, “Cardioprotective effects of curcumin-loaded magnetic hydrogel nanocomposite (nano-curcumin) against doxorubicin-induced cardiac toxicity in rat cardiomyocyte cell lines,” Artificial Cells, Nanomedicine, and Biotechnology, vol. 45, no. 4, pp. 731–739, 2017.

[40] M. I. Qadir, S. T. Naqvi, and S. A. Muhammad, “Curcumin: a polyphenol with molecular targets for cancer control,” Asian Pacific Journal of Cancer Prevention, vol. 17, no. 6, pp. 2735–2739, 2016.

[41] L. Huan, L. H. Liang, and X. H. He, “Role of microRNAs in inflammation-associated liver cancer,” Cancer Biology & Medicine, vol. 13, no. 4, pp. 407–425, 2016.

[42] K.-W. Noh, J. Park, and M.-S. Kang, “Targeted disruption of EBNA1 in EBV-infected cells attenuated cell growth,” BMB Reports, vol. 49, no. 4, pp. 226–231, 2016.

[43] N. S. Srivastava and R. A. K. Srivastava, “Curcumin and quercetin synergistically inhibit cancer cell proliferation in multiple cancer cells and modulate Wnt/β-catenin signaling and apoptotic pathways in A375 cells,” Phytomedicine, vol. 52, pp. 117–128, 2019.

[44] Z. Zhao, C. Li, H. Xi, Y. Gao, and D. Xu, “Curcumin induces apoptosis in pancreatic cancer cells through the induction of forkhead box O1 and inhibition of the PI3K/Akt pathway,” Molecular Medicine Reports, vol. 12, no. 4, pp. 5415–5422, 2015.

[45] H. Hu, M. Tian, C. Ding, and S. Yu, “The C/EBP homologous protein (CHOP) transcription factor functions in endoplasmic reticulum stress-induced apoptosis and microbial infection,” Frontiers in Immunology, vol. 9, p. 3083, 2019.