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Journal of Nutritional Biochemistry 41 (2017) 124–136

Journal of Nutritional Biochemistry

Quercetin induces apoptosis and autophagy in primary effusion lymphoma cells by inhibiting PI3K/AKT/mTOR and STAT3 signaling pathways☆

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Received 17 June 2016; received in revised form 23 November 2016; accepted 28 December 2016

Abstract

Quercetin, a bioflavonoid contained in several vegetables daily consumed, has been studied for long time for its antiinflammatory and anticancer properties. Quercetin interacts with multiple cancer-related pathways such as PI3K/AKT, Wnt/β-catenin and STAT3. These pathways are hyperactivated in primary effusion lymphoma (PEL), an aggressive B cell lymphoma whose pathogenesis is strictly linked to the oncogenic virus Kaposis' Sarcoma-associated Herpesvirus (KSHV). In this study, we found that quercetin inhibited PI3K/AKT/mTOR and STAT3 pathways in PEL cells, and as a consequence, it down-regulated the expression of the prosurvival cellular proteins such as c-FLIP, cyclin D1 and cMyc. It also reduced the release of IL-6 and IL-10 cytokines, leading to PEL cell death. Moreover, quercetin induced a prosurvival autophagy in these cells and increased the cytotoxic effect of bortezomib, a proteasomal inhibitor, against them. Interestingly, quercetin decreased also the expression of latent and lytic KSHV proteins involved in PEL tumorigenesis and up-regulated the surface expression of HLA-DR and calreticulin, rendering the dying cells more likely detectable by the immune system. The results obtained in this study indicate that quercetin, which does not exert any cytotoxicity against normal B cells, may represent a good candidate for the treatment of this aggressive B cell lymphoma, especially in combination with autophagy inhibitors or with bortezomib.

Keywords: Quercetin; PEL; KSHV; mTOR/PI3K/AKT; STAT3; Il-6

1. Introduction

Primary effusion lymphoma (PEL) is an aggressive B cell lymphoma whose pathogenesis is strictly linked to KSHV, an oncogenic virus belonging to the gammaherpesvirus family and often associated also with Epstein Barr virus (EBV). PEL cells relay for their survival and growth on the activation of several pathways such as Signal Transducer and Activator of Transcription 3 (STAT3), Phosphatidylinosittide 3-kinase/Protein kinaseB/mammalian target of Rapamycin,(PI3K/AKT/mTOR), Wnt/β-catenin and Nuclear Factor Kappa B (NF-kB) [1–4]. Thus, their inhibition has the potential to reduce cell proliferation and/or induce apoptosis in PEL cells [5–7]. These pathways are activated by the expression of KSHV viral proteins such K1, G protein-coupled receptor (vGPCR), latency-associated nuclear antigen (LANA) and FADD-like interleukin-1 beta-converting enzyme inhibitory protein (vFLIP) [8,9]. They are also activated by cytokines released by PEL cells, such as Interleukin 6 (IL-6) and Interleukin 10 (IL-10), which act in an autocrine fashion, promoting cell survival [10,11]. A positive feedback loop exists in which the expression of viral proteins activates signaling pathways that induce the release of cytokines that, in turn, amplify the activation of the same pathways. Therefore, these cytokines play a fundamental role in pathogenesis of KSHV-associated diseases and targeting them may represent, *per se*, a promising therapeutic strategy against PEL [10]. Another effective therapeutic approach aimed at inducing cell death in PEL, as well as in other cancers displaying multiple pathway activation, is to concomitantly target several activated pathways or the multiple kinases involved in those. This strategy could avoid the occurrence of feedback loops which could occur when using a single-target agent, as in the case of rapamycin that inhibits mammalian target of Rapamycin complex 1 (mTORC1) and induces the phosphorylation of Akt at Ser473 *via* the rapamycin-insensitive mammalian target of Rapamycin complex 2 (mTORC2) [12]. Differently from rapamycin, NVP-BEZ235, which inhibits both mTOR and PI3K, hampers this feedback loop and exerts a stronger cytotoxic effect against PEL [13]. Quercetin, the most abundant flavonoid found in fruits, vegetables and in beverage such as tea and red wine [14], has been reported to function as a dual mTOR

☆ Funding sources: This work was supported by ASI (Italian Space Agency) (2014-033-R.O.).
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http://dx.doi.org/10.1016/j.jnutbio.2016.12.011
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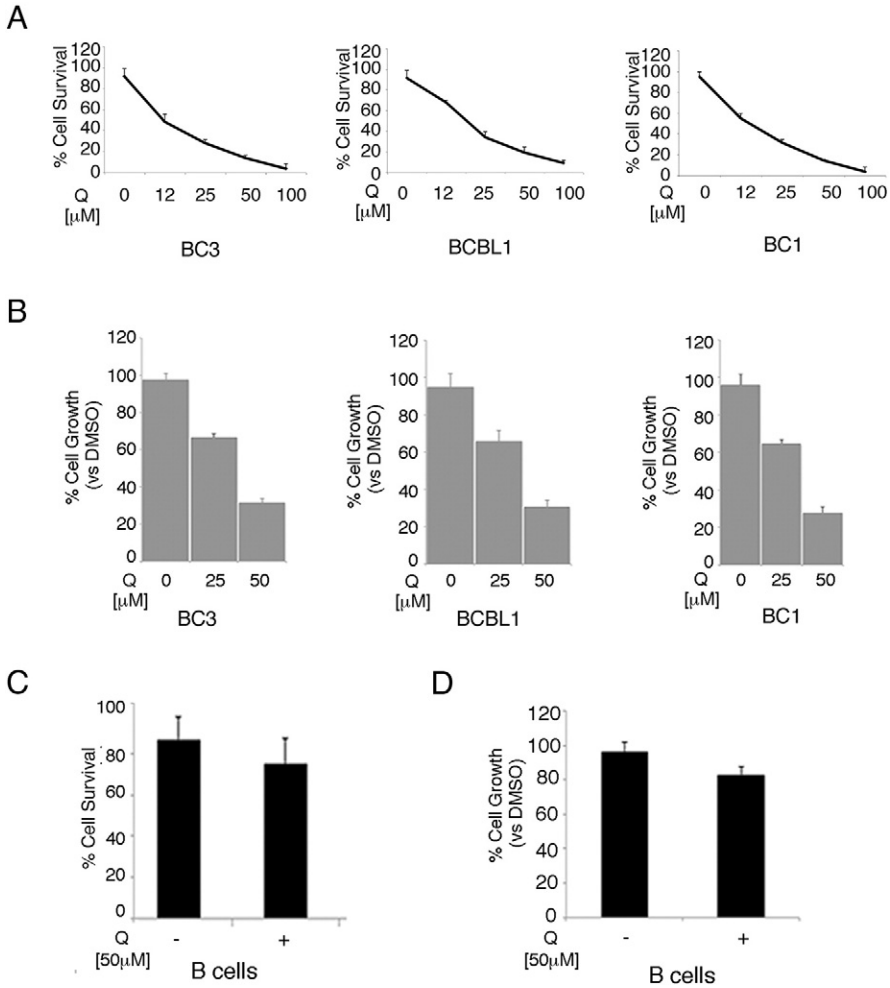

Fig. 1. Quercetin impairs PEL cell survival. (A) Quercetin-induced loss of viability in PEL cell lines is a dose-dependent manner. BC3, BCBL1 and BC1 cells were treated for 24 h with different quercetin (Q) concentrations (from 12 to 100 μM). Cells were counted by trypan blue exclusion assay, and the mean of the percentage of cell survival plus S.D. of five independent experiments is indicated; (B) Cell growth was evaluated by Sulforhodamine B assay. The percentage of cell growth was calculated by normalizing the OD values of quecetin-treated *versus* the DMSO-treated PEL cell lines. The results are expressed as the means ± S.D. of five independent experiments performed in triplicate. (C) B cell viability was evaluated by trypan blue assay and (D) by Sulforhodamine B assay. The mean of cell survival or cell growth plus standard deviation of five independent experiments is indicated.

and PI3K inhibitor [15]. In addition, it can target several prosurvival pathways, for example, Wnt/β-catenin [16,17], whose activation is related to PI3K/AKT/mTOR signaling *via* glycogen synthase kinase (GSK) 3 [18], and STAT3 [19] which is also interconnected with PI3K [20,21]. All these pathways can be also concomitantly inhibited by quercetin in several cancer cell types [22,23]. PEL survival is strongly dependent on all these pathways [3,7,9,24], also because they promote the release of prosurvival cytokines [13,25]. Thus, in this study, we investigated for the first time whether quercetin would target one or more of these pathways in BC3, BC1 and BCBL1 PEL cells and decrease the production of IL-6 and IL-10. On the other hand, the cytokine reduction could interrupt their mediated prosurvival signaling, leading to PEL cell death. The cross talk between the main prosurvival pathways activated in these cancer cells was also investigated. Interestingly, PI3K/AKT/mTOR and STAT3 pathways also play a role in the regulation of autophagy [26,27], a catabolic process basally activated in cancer cells and up-regulated by nutrient depletion or by other stressful conditions. Autophagy can be considered "a double edge sword" since it can either counteract or promote cell death, depending on the different cell types or conditions [28]. Thus, the effects of quercetin on autophagy and the role of autophagy in PEL cell survival were also investigated. Finally, its capacity to increase the cytotoxicity of bortezomib, the immunogenicity of its induced cell death and its effect toward normal B cells, from which PEL cells arise, was explored.

2. Materials and methods

2.1. Cells

The BC3 (American Type Culture Collection, Manassas, VA, USA; ATCC), BCBL1 (kindly provided by Prof. P. Monini, National AIDS Center, Istituto Superiore di Sanità, Rome, Italy) and BC1 (American Type Culture Collection, Manassas, VA, USA; ATCC) cells, human B-cell lines derived from PEL carrying latent KSHV, were cultured in RPMI 1640 (Sigma Aldrich, R0883), 10% fetal bovine serum (FBS) (Sigma Aldrich, F7524), L-glutamine (Aurogene, AU-X0550) and streptomycin (100 μg/ml) and penicillin (100 U/ml) (Aurogene, AU-L0022) in 5% CO_2 at 37 °C.

A stable BC3 cell line expressing GFP-LC3 was grown in complete RPMI medium supplemented with 0.8-mg/ml geneticin/G418 (Life Technologies, 10,131–027).

B lymphocytes were isolated by Fycoll-Paque gradient centrifugation (Cedarlane, CL5020) from buffy coats and selected using CD19 MAb-conjugated magnetic microbeads (Miltenyi Biotec, 130–050-301). B cells were then cultured in RPMI medium plus 10% FBS, L-glutamine, streptomycin and penicillin.

2.2. Cell treatments

PEL cells were treated with quercetin (Sigma Aldrich, Q4951) at 12, 25, 50 and 100 μM for 24 h. Quercetin was dissolved in DMSO solution, and the vehicle (DMSO) was