PERK

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Multiple myeloma is a bone marrow cancer that represents a severe health threat. The drugs used nowadays in chemotherapy often encounter resistance leading to a dramatic loss of their efficacy, which consequently affects patients' survival. Previous studies have shown that the protein kinase R (PKR)-like ER kinase (PERK) pathway, which is one of the three branches of the unfolded protein response, is highly activated in multiple myeloma, possibly contributing to the chemotherapy resistance that these patients develop. In this study, we have used the compound GSK2606414, which is a PERK inhibitor, and found that myeloma cells are highly sensitive to this molecule. These effects were more pronounced when the inhibitor was used in combination with an anti-myeloma drug such as the proteasome inhibitor bortezomib, suggesting that the PERK pathway could be a potential therapeutic target for the treatment of multiple myeloma patients.

PERK MULTIPLE MYELOMA BORTEZOMIB SURVIVAL APOPTOSIS

1. Introduction

PERK is a BiP-bound type 1 transmembrane protein of around 120 kDa with a C-terminal cytosolic domain that possesses serine/threonine kinase activity and an IRE1-like ER luminal domain[1]. Upon accumulation of unfolded proteins in the ER lumen, PERK dimerization and phosphorylation inactivates the general translation initiation factor EIF2α, which is required for the 80S ribosome assembly, resulting in a general shutdown of polypeptide synthesis [2]. Although phosphorylation of eIF2α inhibits general mRNA translation initiation, it is required for the selective translation of several mRNAs such as those encoding activating transcription factor 4 (ATF4) [3][4][5]. ATF4 is a transcription factor in the cAMP-response element binding (CREB) family and activates many genes involved in controlling the UPR including chaperones such as BiP and GRP94, genes involved in suppressing oxidative stress, as well as genes involved in amino acid metabolism and transport [6]. ATF4 also induces the expression of the CCAAT/enhancer-binding protein (C/EBP) homologous protein (CHOP or GADD153) and the marker of ER stress-induced apoptosis growth arrest DNA damage 34 (GADD34) [7][8][9].

2. Role of PERK

The role of PERK activation in cell proliferation and apoptosis has been extensively studied in various tumors [10][11][12]. PERK has been shown to display a protective role, but it can also induce cell death mechanisms due to prolonged activation, suggesting that further investigation on its specific role is needed. Specifically, it has been

found that, in addition to promoting survival, PERK can also suppress tumor growth of advanced carcinomas such as squamous and colorectal carcinoma cells[10]. In other studies, it was found that the integrated stress response coordinated by PERK can both promote and inhibit medulloblastoma tumorigenesis by regulating apoptosis [11][12]. In addition, activation of PERK has been implicated in a wide variety of cancers, as it enhances responses to chemotherapy[13][14][15][16][17][18][19], while knockdown of PERK in MM cells resulted in autophagic cell death [20]. Moreover, in a bortezomib (BTZ)-resistant subpopulation of myeloma cells, it was found that BTZ resistance can be reversed by eIF2α inhibition [21].

Developing agents that would successfully target components of a signaling pathway associated with the precise pathogenic molecular driver for each individual cancer is the key to effective molecular therapies. These pathways are often part of oncogenic networks whose effective inhibition would sensitize tumor cells and successfully drive them to apoptosis and abrogation of the malignant state. The development of pharmacological tools that selectively modulate a druggable enzyme greatly facilitates the investigation of the protein's biological function and potential therapeutic application. UPR signaling has been shown to play a fundamental role in the development and regulation of tumors, particularly those with a secretory phenotype such as MM. Several studies using animal models have shown that manipulating various UPR proteins by gain- or loss-of-function genetic interventions can trigger antitumor effects [22][23]. PERK is one of the main primary UPR effectors and the development of PERK inhibitors has been of particular interest, since recent evidence implicates PERK as a contributor to initiation and progression events in cancer as well as a supporter of cancer resistance to chemotherapy [3][24]. Therefore, inhibiting PERK in cancer cells may suppress their ability to adapt in stress conditions, leading to apoptosis and/or tumor growth inhibition. GSK2606414 has been characterized as one of the first-in-class selective PERK inhibitors identified from a kinase inhibitor library. GSK2606414 has been found to be highly potent for inhibiting PERK in vitro with an IC_{50} dose of lower than 1 nM and has also shown the inhibition of tumor growth in a human tumor xenograft in mice [25][26].

The study reports the anti-myeloma effects of GSK2606414 in human myeloma cells. Initially, our study revealed that PERK is expressed in myeloma cells, as demonstrated by the expression patterns of our panel of myeloma cell lines as well as of $CD138^+$ plasma cells isolated from selected myeloma patients' bone marrow samples. In a previous study, it was shown that knockdown of PERK resulted in an autophagic cell death response, suggesting that PERK activation is a necessary element for the metabolic transformation of a plasma cell to a myeloma cell, but also as an impediment of the apoptotic response, revealing the dual activity of PERK, as both a protector and a cell death promoter in MM [14]. Given the expression profile of PERK in myeloma cells, Researchers showed that treatment with GSK2606414 resulted in a dose-dependent anti-proliferative outcome in eight myeloma cell lines. Interestingly, myeloma cell lines with high expression levels of PERK showed increased sensitivity to the PERK inhibitor, and demonstrated lower IC_{50} values and faster responses, highlighting the on-target effects of the selective inhibitor. Additionally, PERK inhibition prompted high apoptotic effects in the four most highly PERK-expressing myeloma cell lines, with an increased apoptotic signal of 8–30% compared to non-treated cells, in agreement with the results of the WST1 assay. These effects were coupled with the suppression of the main molecular targets of PERK such as ATF4, EIF2α and phospho-EIF2α, but not CHOP.

Interestingly, our results showed that PERK inhibition also slightly affected the pro-survival molecules of the two other branches of the UPR, namely XBP1s and ATF6, suggesting that it might also exert an overall anti-UPR inhibitory effect. This was also shown by the total UPR expression profile and post cell-treatment with GSK2606414, which revealed the suppression of major UPR driver genes, including *IRE1* (*ERN1*) and *XBP1*, which are key players in MM tumor growth [27][28]. In addition, treatment of MM cells with GSK2606414 downregulated the *CEBPB* gene, which regulates transcription factors critical for the proliferation and survival of MM cells [29], and the *PPP1R1A* gene, which is upregulated by ATF4 and provides a negative feedback loop by dephosphorylating eIF2A [30]. On the other hand, MM cell exposure to GSK260614 upregulated genes such as *HSPA2* and *HSPA1B*, which are members of the heat-shock protein family and studies have shown that breast cancer patients overexpressing *HSPA2* exhibited longer survival [31]. In addition, treatment of MM cells with GSK2606414 resulted in the upregulation of *HERPUD1* gene expression, which is an ER stress responsive gene that was previously connected to reduced tumor-associated expression in prostate cancer cells [32]. Across the genes differentially expressed upon treatment with GSK260641, there are several genes that are directly associated with the PERK pathway such as *DDIT3* and *CEBPB* through *ATF4*, while most genes are indirectly connected to the PERK pathway, such as *HEPPUD1*, which is regulated by the ER stress-specific branch of the UPR [33]. As with the IRE1-XBP1 pathway, which is a key pathway in MM biology, authors hypothesized that, through the inhibition of one of the three branches of UPR, cells are in a process of adaptation to the changes that might occur by the suppression of PERK-related genes, activating other branches of the UPR by the positive or negative regulation of UPR-related genes. In contrast to PERK inhibition, although *PERK* knockdown suppressed the expression of ATF4 as well as of total and phosphorylated EIF2α, it differentially affected the expression levels of ATF6 and XBP1 in the two MM cell lines assayed, indicating a differential mechanism of action in the supporting branches of the UPR such as IRE1 and ATF6.

The activated UPR in plasma cells could potentially suppress cellular sensitivity to pharmacological compounds by emerging resistance to therapeutic agents. Thus, authors investigated whether GSK2606414 exerts synergistic effects with BTZ in MM cells. Our results indeed showed that the combination of GSK2606414 and BTZ led to increased apoptosis and decreased cell survival compared to solely BTZ-treated cells. To further evaluate the apoptosis phenotype seen by the Annexin/PI technique, authors screened these samples with an array detection system, which detects 35 human apoptosis-related proteins. Our results showed that the Annexin/PI phenotype was also coupled with the over-expression of many apoptotic proteins when (a) GSK2606414-treated cells were compared with non-treated cells, as well as when (b) GSK2606414/BTZ-treated cells were compared to BTZ-treated cells. Given the dual function of PERK both in cell survival and apoptosis[10][34], authors sought to determine whether GGSK2606414 on its own or, more interestingly, in combination with BTZ, would drive cells to increased apoptosis and hence sensitize cells to more effective combination therapies. Among the apoptotic proteins upregulated, survivin has been previously described as being associated with ER stress and UPR gene expression in colonic epithelial cells[35]. Furthermore, claspin has also been previously reported as a negative regulator of DNA replication inhibited by PERK [36]. Finally, several highly upregulated proteins such as TRAIL, BAD and HTRA2 have been extensively studied as being apoptotic drivers in MM [37][38][39][40][41][42].

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