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P351 PROTEASOME INHIBITORS INDUCE DNA DAMAGE AND MITOTIC CATASTROPHE IN ACUTE LYMPHOBLASTIC LEUKAEMIA (ALL) VIA AUTOPHAGY-MEDIATED DEGRADATION OF WEE1

Topic: 1. Acute lymphoblastic leukemia - Biology & Translational Research

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Background:

ALL is an aggressive haematolymphoid malignancy and the prognosis is poor in adults with long-term survival of only 30% due to the high prevalence of high-risk subtypes. Therefore, there is an urgent need to develop novel treatment for ALL. Carfilzomib is a second-generation proteasome inhibitor which is more potent than its first-generation counterpart, bortezomib, with an excellent activity against bortezomib-resistant plasma cell myeloma. However, pre-clinical and clinical studies of carfilzomib in ALL are limited. Targeting DNA damage and mitotic defects of cancer cell is a major treatment strategy of cancer. WEE1 kinase prevents mitosis of cells with unrepaired DNA through inhibiting cyclin B. Pharmacological inhibition of WEE1 was effective in treating T-ALL with induction of DNA damage. Thus, targeting WEE1 kinase is an attractive approach of novel therapy in ALL. However, there are no studies to evaluate the mechanism of action of carfilzomib in ALL, especially in the aspect of DNA damage and mitotic catastrophe. Moreover, the role of WEE1 modulation by carfilzomib and its underlying mechanism has not been studied in ALL.

Aims:

In view of these unmet clinical needs and research gap, we conducted the study with the aims: 1). To show that carfilzomib induces DNA damage and mitotic catastrophe; 2). To show that carfilzomib induces WEE1 downregulation and delineate underlying mechanisms.

Methods:

Cell lines representing high-risk ALL were used namely TOM-1, SEM, LOUCY, CCRF-CEM, KE-37 and PEER. They were treated with various dose of carfilzomib. Cell viability was assessed by trypan blue assay and apoptosis was assessed by flow cytometry study of double staining for annexin V and PI, after treating the cells for 24 and 72 hours. Comet assay was used to assess DNA damage. Western blot analysis of WEE1, LC3-I and LC3-II were performed. Quantitative PCR for ER-stress related genes were done. Finally, fluorescence microscopy was performed to assess mitotic catastrophe.

Results:

Cell viability was reduced, and percentage of apoptotic cells was increased in a time and dose-dependent manner upon treating with carfilzomib for 24 & 72 hours. Comet assay showed evidence of DNA damage after treating with carfilzomib for 24 hours. Fluorescence microscopy also demonstrated evidence of mitotic catastrophe upon treating with carfilzomib. WEE1 was downregulated upon treatment of carfilzomib for 24 hours, with evidence of autophagy induction as shown by western blot analysis of LC3-1 and LC3-II. Upregulation of ER-stress related gene, CHOP was noted while the mRNA level of WEE1 remained unchanged. Co-treatment of carfilzomib with autophagy inhibitor, bafilomycin A1, reversed the process of WEE1 downregulation.

Summary/Conclusion:

Herein, we proved that carfilzomib induced DNA damage and mitotic catastrophe with WEE1 downregulation.

Moreover, carfilzomib trigger autophagy-mediated degradation of WEE1 in ALL via induction of ER-stress. The findings provide mechanistic insights of carfilzomib in ALL and rationalize the introduction of novel approach of combination treatment of carfilzomib with other DNA damaging agents.

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