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POSTER ABSTRACTS

651.MULTIPLE MYELOMA AND PLASMA CELL DYSCRASIAS: BASIC AND TRANSLATIONAL

Unraveling Antioxidative Metabolic Pathways in Multiple Myeloma: Augmenting IMiD Sensitivity through Intracellular Cysteine Biosynthesis Inhibition

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Introduction

Multiple myeloma (MM) patients often become unresponsive to immunomodulatory drugs (IMiDs) over time. Research has indicated that the cellular ability to counteract oxidative stress plays a crucial role in determining the sensitivity to IMiDs in MM. This study aims to comprehend the metabolic pathways responsible for MM's antioxidative capability and develop new treatments to augment the effectiveness of IMiDs.

Methods

We experimented using MM cells to investigate how different nutrient conditions affect cell proliferation and drug response. We utilized stable isotope-labeled amino acids and mass spectrometry to analyze the metabolites in these cells. We evaluated cell viability through the MTT assay, the CellTiter-Glo® Luminescent Assay, and direct cell counting. Furthermore, we conducted Western blots to measure the levels of various proteins expressed in MM cells grown under different nutrient conditions. To create CRBN knockout cells, we employed the CRBN CRISPR/Cas9 KO plasmid, and for the overexpression of CRBN in MM cells, we used a lentivirus system.

Results

Our research findings reveal that the production of cellular cysteine enhances the cell's ability to counteract oxidative damage, leading to resistance to IMiDs even with the presence of the wild-type CRBN protein. However, MM cells not producing enough cysteine rely on consuming extracellular cystine, which increases sensitivity to lenalidomide and reduces antioxidative capacity. Lenalidomide-sensitive cells acquire more cystine from the extracellular environment than resistant cells. The analysis of cysteine uptake in various MM cell lines using C13-labeled cysteine revealed that the lenalidomide-sensitive cell line, OCIMY5-CRBN, consumed more cysteine from the media, and the presence of L-glutamine further increased this uptake.

Lenalidomide treatment increased the catabolism of cysteine into taurine in sensitive cell lines, confirming the link between lenalidomide's molecular action and cysteine catabolism. Our study also identified that lenalidomide treatment led to an accumulation of CDO protein, which adds molecular oxygen to cysteine, transforming it into cysteine sulfinic acid. Our study suggests that CRBN may play a role in maintaining intracellular CDO protein levels, and the accumulation of CDO protein induced by lenalidomide is the molecular factor responsible for cystine catabolism and sensitivity to IMiDs in multiple myeloma.

We found that by inhibiting intracellular cysteine biosynthesis, we could improve the effectiveness of IMiD against MM. One drug that can achieve this is 5-aza-2-deoxycytidine (DAC), which acts as a DNA methylation inhibitor. Additionally, combining DAC with IMiDs improves their effectiveness, even in cell lines that are resistant to treatment.

Furthermore, we attempted to inhibit cysteine biosynthesis by promoting the synthesis of methionine from homocysteine with the supplementation of 5-methyltetrahydrofolate. We discovered that treatment with 5-methyltetrahydrofolate further enhanced the sensitivity of IMiDs in resistant MM cell lines. Our results confirm that interfering with intracellular cysteine biosynthesis is a promising therapeutic approach for MM and can improve the effectiveness of IMiDs.

Conclusions

The biosynthesis of L-Cysteine is vital for the cellular ability to resist oxidation. Cancer cells that create high levels of cysteine within the cell from methionine generate a more significant amount of reduced cysteine, which can act as antioxidants and make MM cells resistant to IMiDs. On the other hand, cells that cannot produce enough cysteine increase their consumption of extracellular cystine and are more susceptible to IMiDs. Cells that consume an oxidized form of cystine from outside the cell require a reduction process inside the cell, which further reduces their ability to resist oxidation. Additionally, the accumulation of CDO protein induced by lenalidomide can enhance the breakdown of intracellular cysteine and cystine uptake in vulnerable cells. Overall, MM cells that rely on extracellular cysteine are sensitive to IMiDs. Therefore, interfering with intracellular cysteine biosynthesis is a better option for myeloma treatment and enhancing the sensitivity of IMiDs.

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