Unfolded protein response targets chondrosarcoma stem cells via toll like receptor mediated mechanism: exciting therapeutic applications for PRP-1

Victoria Dahl, Andres Ruben Perez, Carina Denny[1], Beatrice Cortina Becker[2], ANIL SEDANI, Daniel Martinez, Aaron Kyle Hoyt[3], Jeffrey Brown, Karina Galoian[4]
[1]University of Miami, [2]Providence Medical Foundation, [3]Loyola University Medical Center, [4]University of Miami Department of Orthopaedic Surg

INTRODUCTION:

Chondrosarcoma is a type of bone sarcoma of mesenchymal origin with a cartilage like appearance and is one of most difficult tumors to diagnose and treat. It is often sporadic but may also originate from osteochondromas or enchondromas. The precise cellular signaling events resulting in this mesenchymal transformation have not been described. Our previous novel work reported for the presence of innate immunity Toll-like receptors (TLR) in human chondrosarcoma cells and demonstrated that the ligand for TLR1/2 and 6 is an antitumorigenic neuropeptide proline rich polypeptide (PRP-1). PRP-1 is an inhibitor of mammalian target of rapamycin complex-1 (mTORC1,) and specifically induces cell death in the cancer stem cell population responsible for disease progression, recurrence of cancer, and resistance to therapies. The exact cellular signaling mechanism by which PRP-targets this cell population is not known.

Neoplasms can be very detrimental to cell integrity due to accumulation of toxic, unfolded proteins. Conditions that alter endoplasmic reticulum (ER) homeostasis functioning generate a state known as ER stress. The cellular response to ER stress is known as the unfolded protein response (UPR) and this response is dependent on cellular and disease context. The UPR is divided into three branches that are triggered by specific ER transmembrane proteins: double-stranded RNA-dependent protein kinase (PERK), Inositol Regulating Enzyme 1 (IRE1), and Activating Transcription Factor 6 (ATF6). Our aim in this study the is to determine the status of UPR in chondrosarcoma and its response to PRP-1 and better understand both in chondrosarcoma cell monolayers and 3D spheroids formed by cancer stem cells.

METHODS:

Cell culture and treatment

2D and 3D cultures of chondrosarcoma JJ012 cells were established. 3D cultures were treated with 20 µg/ml PRP-1, 40 µg/ml PRP-1, and control; 2D cultures were treated with 10 µg/ml PRP-1, 20 µg/ml PRP-1, 40 µg/ml PRP-1, and control.

Protein levels

Protein levels were quantified by western blotting via monoclonal antibodies for 2D and 3D cell lysates from all treatment and control groups and quantified using ImageJ.

mRNA expression

RNA was extracted from 2D and 3D cell lysates from all treatment and control groups. RNA to cDNA reverse transcriptase reactions were run and mRNA expression was determined with RT-qPCR. The results were normalized by the expression of GAPDH mRNA and quantified as the fold-difference compared with that of the control condition.

RESULTS:

Upregulation of mRNA gene expression of ATF4, XBP1, CHOP, IRE1, EiF2A and ATF6 was demonstrated in 2D lysates treated with 10 µg/ml PRP-1 when compared to the untreated control. Additionally, in the 2D lysates upregulation of mRNA gene expression of PERK and ATF6 was demonstrated when comparing the 20 and 40 µg/ml PRP-1 treated groups when compared to the control. In the 3D lysates, upregulation of ATF4, XBP1, IRE1, and PERK was demonstrated in spheroids treated with 20 µg/ml when compared to the control. Upregulation was also shown in the 40 µg/ml treatment groups for ATF4, XBP1, CHOP, IRE1, PERK, EiF2A, and ATF6. These results were also demonstrated in protein levels via Western blotting.

All changes in gene expression were statistically significant by unpaired t-test, and $p < 0.05$ was considered to be statistically significant.

DISCUSSION AND CONCLUSION:

This work demonstrated that in chondrosarcoma UPR response is downregulated. PRP-1 upregulates UPR branches PERK-EiF2A -ATF4-CHOP and ATF6 - IRE1 -XBP1 shown both in protein levels and mRNA expression in both 2D and 3D models of human chondrosarcoma cells. These results also indicate that the PERK -EiF2A-ATF4 -CHOP pathway enhances IRE1-XBP1 signaling. The highest concentration of PRP-1 treated used (40 µg/ml) demonstrated a statistically significant increased in all ER stress proteins in the 3D culture, and the lower concentration treatment of PRP-1 (10 µg/ml) was shown to cause a significant increase in six of the seven ER stress proteins analyzed in the 2D culture lysates.

In this work we presented the experimental evidence of the activation of UPR branches though innate immunity TLR ligand PRP-1, and we hypothesize that this plays a role in the decrease of human chondrosarcoma stem cell population along with epigenetic factors. These results add to the existing evidence for the mechanism through which PRP-1 has tumor suppressing effects and is the first demonstration of how the antitumor UPR response is triggered by innate immunity receptors in human chondrosarcoma tissue. Future direction for this research includes understanding the

specific epigenetic and molecular events connecting UPR and cancer stem cells death. We plan to create human chondrosarcoma xenografts in mice to explore therapeutic effect of PRP-1 in vivo as the next step for this work.