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Investigating the role of the Unfolded Protein Response in Quiescence

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INTRODUCTION

The endoplasmic reticulum (ER) is the entry point for proteins into the secretory pathway. Secretory proteins are co-translationally imported into the ER of the cell, where they are folded, modified, and sent to the Golgi apparatus. ER stress occurs when misfolded proteins overwhelm the capacity of the ER, resulting in activation of the unfolded protein response (UPR). If the number of misfolded proteins exceeds the folding capacity of the ER, the UPR is activated to help balance the protein load of the ER (Walter & Ron, 2011).

Perk, an ER transmembrane kinase and effector of the UPR, phosphorylates the eukaryotic translation initiation factor 2A (eIF2α), leading to temporary stalling of translation and a decreased load on the ER. In addition to its role in the UPR, we are interested in Perk's role in the temporary exiting from the cell cycle, known as quiescence (Eliazer & Brack, 2016). We find evidence for Perk activation during contact inhibition and quiescence but no activation of the other UPR signal transducer, Ire1. Additionally, we find that PERK is required for cells to re-enter the cell cycle. This data indicates that Perk may play a crucial role in entering and exiting of the cell cycle.

RESULTS AND DISCUSSION

Previous work has indicated that Perk plays an important role in regulating quiescence (Ranganathan, et al. 2008). We hypothesized that Perk would be activated during quiescence. To test this hypothesis, mouse pre-osteoblast cells were grown to contact inhibition. Western blot quantification of eIF2α phosphorylation, the downstream target and indicator of Perk activation, indicate increasing phosphorylation during quiescence. These results indicate that Perk is increasingly active during contact inhibition and quiescence.

To ensure that the UPR signal transducer Ire1, an ER transmembrane nuclease, was not activated, we looked at splicing of its downstream mRNA target, X-box binding protein 1(XBP1). Upon activation during ER stress, Ire1 cleaves a 26 base-pair region from XBP1 mRNA. Once cleaved, the mRNA fragments are ligated together to form a spliced product that can be translated into an active transcription factor, turning on a host of genes that increase the folding capacity of the ER and the capacity of the secretory pathway. We collected and purified total RNA from contact inhibited mouse pre-osteoblast cells and utilized gel electrophoresis to observe XBP1 splicing. Gel quantification indicates that there is no change in splicing of XBP1, suggesting that Ire1 is not activated during contact inhibition and quiescence.

Our data led us to investigate the significance of Perk's role in entering and exiting the cell cycle. Perk knockout cells were grown to contact inhibition and quiescence. When re-plated, the cells did not re-enter the cell cycle and resume a normal doubling every 24 to 48 hours. Interestingly, Ire1 knockout cells were able to re-enter the cell cycle similar to wild type after re-plating. These data indicate that Perk is required for re-entry to the cell cycle.

My results suggest that Perk is the UPR signal transducer that is activated during contact inhibition and quiescence. Additionally, Perk appears to be required for re-entrance to the cell cycle. This work sheds light on how mouse pre-osteoblast cells regulate the cell cycle. We are currently working to corroborate these results by stably transfecting Perk back into the Perk knockout cells and monitoring the number of cells during contact inhibition and re-plating.

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