

During tumor development and progression, transformed cells adapt to increased demand on protein and lipid production by enhancing endoplasmic reticulum (ER) function and expansion. To accomplish this, cancer cells take advantage of the multifunctional signaling pathway called the unfolded protein response (UPR). The function of the UPR is to promote cell survival and to restore proper ER function, or alternatively during irrevocable stress, to trigger cell death. Interestingly, although activation of UPR is a common feature of cancer microenvironment, cancer cells have the ability to avoid the UPR-induced transition to apoptosis, and therefore strategies that inhibit these survival pathways have become an attractive target for novel anticancer therapies. Although the UPR provides many appealing therapeutic candidates, IRE1 activity has been a major focus since it promotes a tumoral phenotype in several cancers and furthermore, elevated levels of IRE1 are associated with poor cancer prognosis. Notably, IRE1 splices an inactive unspliced form of XBP1 to a highly active prosurvival spliced XBP1 (XBP1s) transcription factor. XBP1s's function is to enhance the expression of ER-resident chaperones and promotes ER expansion. Notably, however, IRE1 cleaves other mRNAs localized to the ER membrane through regulated IRE1-dependent decay (RIDD). This activity can serve both adaptive and apoptotic branches of UPR. Hence, inhibiting IRE1 activity has consequences for both aspects of UPR, and therefore inhibiting IRE1 has the high risk of off-targets effects.

The novel goal of this proposal is to determine the significance of the XBP1s signaling during cancer cells fate decisions and to define the mechanisms responsible for the XBP1s transcript abundance that could be used to develop novel therapy targets. Based on our preliminary data, our hypothesis is that XBP1s-dependent genes effectively protect cancer cells from stress-induced death and therefore preventing XBP1s accumulation, while not impairing other IRE1 activities could create basis for novel, safer cancer therapies. The rationale for this hypothesis is that our preliminary data indicate that during stress, unspliced XBP1 (XBP1u) is more stable than XBP1s mRNA. Consequently, during stress, the availability of the more stable XBP1u transcript will determine the extent of XBP1s signaling. Therefore, defining and understanding the specific mechanism, responsible for this phenomenon could allow for the decrease of XBP1s levels and thus contribute to development of novel therapeutic approaches.

Finally, the IRE1 mediated splicing of XBP1u relies on the removal of 26 nt sequence from this transcript to produce XBP1s mRNA. We are convinced that synthetic molecules (morpholinos) directed against the splicing sites in XBP1u mRNA could prevent accumulation of XBP1s transcript in ER stressed cancer cells, without affecting other aspects of IRE1 activity.

1. To test the hypothesis that genes that are XBP1-dependent protect cancer cells from ER stress related cell death. We will determine the specific changes the gene expression profiles during prolonged ER stress. We will select XBP1s-dependent genes and verify their role in cell survival. Finally, we will verify their functionality in wide panel of cancer cell lines that are representative for tumors with deregulated XBP1 expression or IRE1 activity including GBM and triple negative breast cancer.
2. To determine if modulation of XBP1 levels by specific miRNAs can be used to control cell fate during UPR. We will identify the differences in specific miRNAs distributions between XBP1u and XBP1s. Next, following validation, we will determine if these miRNA can be used to modulate the extend of XBP1s signaling and thus alter the cell fate decisions.
3. Finally, as an alternative to IRE1 inhibitors, we will test if XBP1 splicing can be prevented with target masks directed against IRE1-spliced consensus motifs in the XBP1u sequence.

Given that this aspect of UPR pathway has not been examined previously and has potentially far-reaching implications in many types of cancer including GBM the studies proposed herein are timely and significant.