In the human cells, the endoplasmic reticulum is the central organelle required for the synthesis, folding and post-translational modifications of membrane and secretory proteins. The ER is also where lipid synthesis occurs. Dysregulation of ER homeostasis (ER stress) can occur in a number of ways and results in the activation of the multifunctional signaling pathway that regulates gene expression called Unfolded Protein Response.

The UPR primarily serves as a cellular adaptive mechanism that alleviates ER stress by modulating gene expression at multiple levels. When the stress persists or when the recovery mechanisms are defective, however, it leads to cell death.

UPR dysregulation contributes to the pathomechanisms of numerous human diseases including non small cell lung cancer, especially in epithelial cells that are the most likely to suffer from environmental stressors. Therefore, it is critically important to understand the mechanisms deciding the cell fate of the UPR in order to develop novel interventions for treating these disorders. Although considerable progress has been made towards understanding the cellular pathways underlying the UPR, the mechanisms determining cell fate remain poorly understood.

Most of the current information available known involves the use of models that utilize high/non-physiological concentrations of chemical stressors. We suggest, however, that what is really needed to understand this process is a much more physiologically relevant dynamic analysis of the UPR cell fate-related transcriptomic profiles. Furthermore, although the UPR signaling is extensively studied, the consequences of ER stress recovery, and how they affect further cell fate decisions remains unclear.

Notably, our preliminary data clearly show that models using low doses of ER stressors allow for a more clear demarcation of the UPR cell fate deciding stage, and thus provide more physiologically relevant insight into the UPR mechanism as it occurs in vivo.

Furthermore, cells in vivo are often exposed to waves of stress inducing factors such as smoking or smog, and thus gaining information how UPR recovery influences further stress responses and cell fate provides important insight into both cancer and lung pathology development.

Our hypothesis is that despite the fact that some mRNAs/miRs have clear adaptive or apoptotic functions during the UPR, the output of the cell fate decision results from complex dynamic changes in the mRNAs and miRs networks that modulate both arms of UPR. Furthermore, despite restoring ER homeostasis, some aspects of the UPR-related signaling pathways remain active at reduced levels, but still influence cell homeostasis and low-level stress responses.

The remaining changes in specific adaptive mRNAs levels may provide a mechanism to protect the cells from the next wave of stress, much like that described for ischemic preconditioning of the heart. In other words, a mild stress preconditions cells to a stronger subsequent stress.

Consequently, this proposal first focuses on applying "mild stress" models to identify the dynamic and specific changes in mRNA and miRs expression networks and to define the mechanisms by which these "dynamic" changes control the UPR-associated cell fate decision. Next, we will follow the transcriptomic consequences of ER stress recovery to determine its effect on cell preconditioning and cell death.

To test these hypotheses, two specific aims are proposed. Aim 1. To test the hypothesis that novel dynamic changes of mRNA/miRNA networks are responsible for the cell fate during the UPR

Aim 2. To test the hypothesis that following a mild ER stress recovery, some factors' expression will be elevated and will precondition cells to subsequent ER stress responses that will alter cell fate decisions.

The significance of the studies in Aim 2 is that we are determining if there is preconditioning or not in airway epithelial cells after an initial stress response. If there is preconditioning as our preliminary data suggests, i.e., stably elevated levels of BiP, then understanding the time course of this preconditioning (when does it occur and how long does it occur?) would be extremely valuable in promoting cell viability (smoke treated airway epithelial cells (COPD model) or promoting cell death (lung cancer cells) through manipulating the miRs/mRNA networks.