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The Survivin and cIAP1 anti-apoptotic proteins are differentially downregulated in response to endoplasmic reticulum stress

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Abstract

The endoplasmic reticulum (ER) is an organelle tasked with synthesis and transport of 90% of new cellular proteins. Dysfunction within this organelle creates signals for repair, adaptation, and in severe cases, cellular apoptosis. Multiple human diseases have been associated with ER dysfunction, and the activation of apoptosis in important populations of cells. Inhibitor of Apoptosis (IAP) proteins are cytosolic proteins that play an anti-apoptotic role in the cytosol. The relationship between endoplasmic reticulum (ER) stress and the expression/stability of IAPs is not well characterized. The objective of this study was to characterize the effect of ER stress on the expression/stability of these members of the IAP family, XIAP, cIAP, Survivin, and Livin.

We also assessed how inhibition of the PI3K/Akt pathway affects expression of these proteins. In model cell lines (BHK21, A549), Survivin and cIAP expression was upregulated in the presence of ER stress. ER stress was shown to induce a reduction of both Survivin and cIAP in a time and dose dependent manner, with Survivin displaying a more dynamic response. The phosphatidylinositol-3 kinase (PI3K) pathway has been associated with regulating expression of some IAP proteins. Inhibition of the PI3K decreased Survivin expression in both cell lines. Further research is required to confirm the effects of ER stress upon regulation of IAP expression (PI3K) and upon stability.

Introduction

The endoplasmic reticulum (ER) is a key organelle within eukaryotic cells that is the site of protein synthesis and modification [5]. Altered intracellular calcium levels, oxidative stress, or viral infections are stress factors that can alter ER homeostasis and lead to the accumulation of misfolded proteins. The unfolded protein response (UPR), a set of complex pathways part of an adaptive measure, is activated to restore homeostasis [5].

Three transmembrane proteins, PERK, ATF6, and IRE1, serve as ER stress sensors involved in the activation of the UPR. Binding immunoglobulin protein (BiP) unbinds from the transmembrane proteins to refold misfolded proteins. This activates several pathways leading to transient arrest of protein translation, degradation of proteins through the proteasome, and the upregulation of chaperones in an effort to rescue the cell by preventing programmed cell death, known as apoptosis [5].

In addition to the UPR, a parallel cell survival mechanism involving Akt kinase, the Extracellular regulated Kinase (ERK) pathway, and cytotoxic inhibitor of apoptosis proteins (IAPs) has also been identified. A baculovirus AIP domain that promotes binding with, and regulation of, pro-apoptotic caspases is conserved in IAPs [4]. By inactivating and ubiquitinating caspases, IAPs work alongside the UPR to regulate the apoptotic response in mammalian cells [2,3]. Prolonged ER stress exhausts the protective measures of the cell. If the UPR cannot regulate the amount of unfolded proteins to restore homeostasis, a caspase cascade is activated resulting in apoptosis [1].

Previous studies have shown increased cell susceptibility to apoptosis, and inhibition of proliferation in cancer cells as a result of IAP downregulation [4,8]. While it is known that altering IAP expression affects the cell’s susceptibility to apoptosis, the impact of ER stress on IAP expression in relation to apoptosis is not fully understood. As abnormal expression of IAPs has been linked to numerous malignancies such as breast and degenerative disorders, therapeutic options may benefit from elucidation of the relationship between ER stress and IAP expression.

The objective of this study was to characterize the effect of ER stress on the expression/stability of five members of the IAP family: XIAP, cIAP, cIAP1, Survivin, and Livin. Data from our lab shows distinct responses of the cell with varying degrees of ER stress. A protective response is observed in cell’s facing low ER stress; cells can be rescued and have a reduced apoptotic response compared to cell’s managing high ER stress. Ultimately we want to assess how intensity of ER stress affects the expression of these proteins, how these proteins are involved in the protective response against low ER stress, and if IAP expression is regulated through the Akt pathway in the ER stress response.

Results

Figure 1. Tunicamycin induces ER stress and an apoptotic response in BHK and A549 cells. (A) BHK21 and A549 human alveolar lung cells were treated with Tunicamycin, a known ER stressor, which prevents proper folding of proteins by stopping N-linked glycosylation. A375M fibroblast cells are a well established model for ER stress because of their robust response. (B) A549 cells were treated with 20nM Tunicamycin (Tuni). (b) untreated (a) and treated with 200nM Tuni (b) shows an apoptotic response in A549 cells. (B) A549 cells are a well characterized epithelial alveolar cancer cell line that also show a response to ER stress though not as pronounced as the response in BHK21. [7]

Figure 2. Survivin and cIAP are the proteins of the IAP family with detectable expression in BHK21 and A549 cells. BHK21 and A549 cells were treated with various doses of Tuni for 24 hours. Expression of cIAP1, cIAP2, Livin and Survivin were analyzed through western blotting. In both cell lines, cIAPs and Survivin expression was shown.

Figure 3. Elevated levels of ER stress and prolonged exposure causes decreased expression/stability of Survivin in BHK21 and A549 cells, and reduced expression of cIAPs in BHK21 cells. BHK21 cells and A549 were treated with various doses of Tunicamycin for 24 hours and analyzed by immunoblot. (A) With greater intensity of, and longer exposure to, ER stress, there is a downward trend of Survivin and cIAPs in BHK21. Survivin has a more dynamic response to ER stress in BHKs. (B) While Survivin displays a modest downregulation induced by elevated levels of ER stress, cIAP expression remains relatively steady. (C,E) Quantitative analysis of Survivin and cIAP expression in BHKs. (D,F) Quantitative analysis of Survivin and cIAP expression in A549. These are observations from two replicates; additional replicates will be completed to confirm findings and determine statistical significance.

Discussion

IAPs

IAPs are a superfamily of proteins that are involved in the regulation of apoptosis. They are known to bind to and inactivate caspases, which are the executioners of apoptosis. XIAP is the most well-studied IAP and is involved in the regulation of various cellular processes, including cell proliferation, differentiation, and stress response. It is known to be activated by various stressors, including ER stress, and plays a crucial role in the survival of cells during stress.

Survivin

Survivin is a member of the IAP family and is known for its anti-apoptotic properties. It is involved in the regulation of apoptosis and has been associated with the regulation of cell proliferation, cell cycle progression, and genomic stability. Survivin expression is known to be induced by various stressors, including ER stress, and has been linked to various diseases, including cancer.

Conclusion

• In BHK21 and A549 cells Livin, XIAP and cIAP2 expression is not detected.
• ER stress induced by TUNI causes downregulation of Survivin and cIAP1 expression/stability in a time and dose dependent manner in BHK21 cells
• In BHK21 cells, Survivin has a more dynamic response to ER stress
• In A549 a downward trend of survivin is observed in response to greater ER stress, while cIAP1 expression remains relatively stable.
• The PI3 kinase pathway is involved in regulating IAP expression/stability in the ER stress response in BHK21 and A549 cells.

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Works Cited