Assessment of p38 and extracellular signal-regulated kinase (ERK) in regulating apoptosis during low and high ER stress

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The endoplasmic reticulum (ER) is an organelle within the cell that functions in the folding of proteins, storage of calcium (Ca^{2+}), oligosaccharide synthesis, lipid metabolism, and drug detoxification in certain types of cells. When the ER is undergoing stress, its protein folding capabilities are compromised. When this stress on the ER occurs, the unfolded protein response (UPR) is initiated. The UPR has several different pathways by which it can either send adaptive signals or signals that initiate programmed cell death, also known as apoptosis, to the nucleus for the ultimate degradation of the cell. However, ER stress has also been shown to activate ERK, at either low or high ER stress, leading to cell proliferation and even survival mechanisms.

Another method of mediation of cellular responses to stress stimuli is via mitogen-activated protein kinases (MAPKs), two of which are MAPK p38 (p38) and MAPK p44/42, or Extracellular signal-regulated kinase (ERK)/12, or ERK. Stress-induced p38 has been shown to play a role in apoptotic signaling, while ERK has been shown to play a role in survival signaling. ERK is activated via a pathway known as the Ras/Raf/MEK/ERK pathway, which is a signal transduction pathway initiated by growth signals on the extracellular membrane. When the ER is stressed, the unfolded proteins, also known as stress proteins, become activated and begin either adaptive or apoptotic responses (UPR) is initiated. The UPR has several different pathways by which it can either send adaptive signals or signals that initiate programmed cell death, also known as apoptosis, to the nucleus for the ultimate degradation of the cell. However, ER stress has also been shown to activate ERK, at either low or high ER stress, leading to cell proliferation and even survival mechanisms.

This investigation will focus on the roles of p38 and ERK in apoptosis during low and high ER stress over time, rather than over different concentrations. This investigation will also assess the roles of these MAP kinases as well as JNK (c-Jun N-terminal kinase), a kinase associated with apoptosis, during increasing levels of ER stress.

**Works Cited**


[4] Osborne, A.D., and Exton, J.H. (2004). Critical Role of ERK induction over time during low and high ER stress. Western blot analysis revealed that p38 increases in a dose-dependent manner, while ERK is only activated at low concentrations Tunicamycin.


**Figure 1. Diagram of Ras/ Raf/MEK/ERK pathway**

ERK is a kinase that is activated by a phosphorylation cascade that often begins outside the cell. However, ER stress has also been shown to activate ERK, at either low or high ER stress, leading to cell proliferation and even survival mechanisms.

**Figure 2. Over increasing concentrations of Tunicamycin, p38 levels increase while ERK levels are transient**

BHK-21 cells were treated with increasing concentrations of Tunicamycin. Western blot analysis revealed that p38 increases in a dose-dependent manner, while ERK is only activated at low concentrations Tunicamycin.

**Figure 3. p38 and ERK induction are not time-dependent in either low or high ER stress in BHK-21 hamster fibroblast cells**

BHK-21 cells were treated with 20nM and 200nM Tunicamycin for time points up to 36 hours and were analyzed by Western blotting. p38 induction is higher at 200nM than at 20nM. There were no significant differences in ERK induction between doses. (3B) Quantification of p38 induction over time and at low and high ER stress. A significant difference was found in p38 induction between doses (p<0.05) but no significant differences were found when comparing time points in 200nM (p>0.05) or in 20nM (p>0.05). (3C) Quantification of ERK induction over time at [1]. Over increased concentrations of Tunicamycin, p38 induction was found between 20nM and 200nM Tunicamycin (p>0.05) or between time points within concentrations (p>0.05). (3D) Quantification of tubulin control across time points during low and high ER stress. No significant differences found between treatments.

**Figure 4. Inhibition of p38 leads to cell rescue up to high levels of ER stress while inhibition of ERK leads to increased cell death at only at 200nM Tunicamycin**

BHK-21 cells were treated with increasing concentrations of Tunicamycin with an assortment of kinase inhibitors and analyzed using Crystal Violet. (4A) Photo of crystal violet assay. (4B) Quantification of p38 inhibition compared to control. Student-t test reveals cell rescue up to 200nM Tunicamycin (p<0.05). (4C) Quantification of JNK inhibition compared to control. No significant differences found (Student-t test: p>0.05). (4D) Quantification of ERK inhibition (with ERK inhibitor II) compared to control. No significant differences found (Student-t test: p>0.05). (4E) Quantification of ERK inhibition (with ERK inhibitor #8) compared to control. Only a significant difference was found between 20nM and 200nM ERK inhibition group and 20nM control group (p<0.05). Two of the three replicates were seeded at 10,000 cells/well; the third replicate was seeded at 12,000 cells/well. Thus, these cannot be treated as true replicates, and future replicates are necessary to validate these conclusions.

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