The Effect of Dominant Negative MKK Expression on pH-induced Increases in PEPCK mRNA Levels

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Abstract:
Acid-base homeostasis is essential for survival. When metabolic acidosis is induced by factors such as prolonged starvation, severe shock, high protein diet, or uncontrollable type 1 diabetes, the kidneys act to compensate for the decrease in pH. Renal carbohydrate metabolism, which is sustained through increased expression of phosphoenolpyruvate carboxykinase (PEPCK) and glutamate dehydrogenase (GAD), is activated during metabolic acidosis. Mammalian target of rapamycin (mTOR) and Akt are thought to play key roles in the signal transduction pathway that lead to the activation of PEPCK and glutamine synthetase. To examine the potential roles of MKK3 and MKK6, LLC-PK1-FBPase+ cells were stably transfected with dominant negative forms of either of both kinases. Expression of the transgenes was controlled by an Tet-responsive promoter element (TRE). Doxycycline (dox) was used to inhibit transcription by preventing the activity of the TRE. Northern blot analysis was also performed to examine the effect of dnMKK expression on levels of PEPCK mRNA in cells grown in both aci- and neutral pH media. The results indicate that the dominant negative forms of MKK3, MKK6, and both kinases (MKK3/6) block the acid-induced increases in PEPCK mRNA.

Introduction:
Metabolic acidosis involves an abnormally high acid load and a low level of bicarbonate in the blood. Generation of bicarbonate by conversion of 

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\text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{H}_2\text{CO}_3 \rightarrow \text{Na}_2\text{CO}_3
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is essential to maintain acid-base homeostasis. The kidney plays a critical role in regulating the acid-base status of the body. The kidney is responsible for excreting excess acid and reabsorbing bicarbonate to maintain a constant pH. This process is known as renal compensation.

During metabolic acidosis, renal carbohydrate metabolism, which is sustained through increased expression of phosphoenolpyruvate carboxykinase (PEPCK) and glutamine synthetase, is activated. This increase in carbohydrate metabolism helps to compensate for the decrease in pH. Renal carbohydrate metabolism is activated to compensate for the decrease in pH and to maintain a constant pH. This process is known as renal compensation.

Figure 1: The proposed signal transduction pathway results in an increase in transcription of the PEPCK gene in response to decreasing pH. MKK3 and MKK6 are thought to play key roles in the signal transduction pathway that lead to the activation of PEPCK and glutamine synthetase. A decrease in pH activates MKK3, which leads to the phosphorylation of p38 and the activation of PEPCK. This process is activated in acidic media. In the presence of Dox (≥20 nM), the IA protein is unable to bind the TRE and transcription is turned off. Western blotting with anti-phospho-p38 antibody indicates that the dominant negative forms of MKK3 and MKK6 are inhibited following removal of dox.

Figure 2: The doiMKK3 construct contains mutations in the residues that are phosphorylated when MKK3 is activated. Amino acids 180 to 185 and 193 to 195 are alanine. The doiMKK3 construct was created by mutation of alanine 82 to alanine within the ATP binding site. A non-essential FLIPase (Ap-Sp-Ap-Sp-Ap-Ap-Sp-Ap-Ap-Ap-Ap) was incorporated into the constructs and the four constructs were then cloned into a pTRE2-plasmid, a vector in which the promoter contains multiple Tet-responsive elements (TREs).

Figure 3: LLC-PK1-FBPase+ cells expressing dominant negative forms of MKK3 and MKK6 from a tet-responsive promoter were developed. In the absence of Dox, the TRE is not activated and the IA protein is unable to bind the TRE. The presence of Dox (+20 nM), the IA protein is unable to bind the TRE and transcription is turned off. Western blotting with anti-phospho-p38 antibody indicates that the dominant negative forms of MKK3 and MKK6 are inhibited following removal of dox.

Figure 4: Levels of endogenous MKK3 and MKK6 in LLC-PK1-FBPase+ cells and LLC-PK1-FBPase+ cells were quantified by western blots. The results indicate the presence of high endogenous levels of MKK3 but low levels of MKK6 in LLC-PK1-FBPase+ cells (Andratsch, Mamoli).

Figure 5: Time course of expression of doiMKK3 in cells grown with (+) and without (-) dox. Western blots were prepared from extracts of doiMKK3 cells grown for 1 to 5 days with or without dox and then probed with anti-FLAG, anti-MKK3, and anti-MKK6 antibodies.

Figure 6: doiMKK3 cells were grown to full confluency for 12 days. 24 hours prior to harvest, cells were treated with pH 7.4 or 6.9 media. RNA was isolated from the cells and Northern blots were performed probing for PEPCK mRNA. The blots were stripped and reblotted with 18S RNA. Panel A: Comparison of the effect of doiMKK3 expression and SB203580 inhibitor on acid-induced increases in PEPCK mRNA. doiMKK3 cells were grown to full confluency for 12 days. 24 hours prior to harvest, cells were treated with pH 7.4 or 6.9 media and 10 mM SB203580 inhibitor was added to cells grown with dox. Northern blots were performed on doiMKK3 cells and the effect of doiMKK3 expression was compared to implementation of SB203580 on cells grown in pH 7.4 and 6.9 media.

Conclusions and Future Directions:
- Either MKK3 and MKK6 are required for signal transduction that leads to increases in PEPCK mRNA in response to treatment with acidic media.
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References:

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Images:
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