Isolation of a Novel Chymase in Mouse Epidermal Keratinocytes after Electrophilic Exposure

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Mustard gas has been used by dictators and terrorists even to today. Despite this, the mechanisms behind mustard induced skin blistering remains a mystery. A target for countermeasures against mustard blistering is the gelatinase, matrix metalloproteinase 9 (MMP9). MMP9 is known to be released into the extracellular matrix as a proenzyme. In humans, the activator for the proenzyme and the specific conditions to release this activator remain unknown. Recently an alpha chymase was discovered in human keratinocytes and was found to be activated by scratch wounding or the electrophile, peroxynitrite. In mice, the functional analogue of human chymase, mMCP4, is known to cleave proMMP9, however this enzyme has thus far not been identified in epithelial cells. We hypothesize that, like humans, a chymase is expressed in mouse epidermal keratinocytes and may be upregulated and activated by oxidative and nitrosative stress after mustard exposure. Mouse epidermal keratinocytes (MEKs) were grown and treated with a peroxynitrite generator, SIN-1. Messenger RNA were isolated from both treated and untreated cells, and converted to cDNA for polymerase chain reaction. Products were detected with a 10% acrylamide gel. Primers made for the two most likely expressed isoforms of mouse chymase in mouse keratinocytes, mMCP-4 and mMCP-5, were used in separate reactions to deduce the specific isoform of chymase. We found that both chymase mMCP-4 and mMCP-5 appear to be expressed in mouse epidermal keratinocytes. Additional studies are necessary to confirm these results and elucidate the role chymase may have in human skin blistering.