# ROLE OF METALLOPROTEINASES IN MEDIATING HEAVY METAL TOXICITY IN THE DOGFISH SHARK, SQUALUS ACANTHIAS

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The matrix metalloproteinases (MMPs) are a closely related family of matrix modifying enzymes thought to mediate tissue breakdown at the sites of injury and repair. MMPs play an important role in mediating the inflammatory response as well as being hypothesized to be important in tissue invasion and metastasis (Matrisian L.M., Trends in Genetics 6: 121-125, 1990; Stetler-Stevenson W.G. et al, Ann. Rev. Cell Biol. 9: 541-73, 1993). Although a role has been hypothesized for these enzymes in renal (Davies M. et al, Kidney International 41: 671-678, 1992) and hepatic (Arthur M.J.P., Seminars in Liver Disease 10: 47-55, 1990) lesions associated with cellular proliferation and fibrosis, surprisingly little data have been obtained regarding the modulation of their activity in the setting of tissue injury.

The activity of MMPs can be postulated to be potentially modulated by heavy metals at both the transcriptional and post-translational level. The transcriptional regulatory pathways of metallothionein and the MMPs show considerable overlap, and the two have been shown to be coordinately regulated in chondrocytes in osteoarthritis (Zafarullah M., et al., FEBS 306: 169-172, 1992). This suggests that heavy metals, which induce metallothionein expression, could also effect MMP gene transcription. Furthermore, collagenases are activated in vitro by organomercurials (Mookhtiar K.A. et al., Analytical Biochemistry 158: 322-333, 1986), indicating that interaction of other metal ions with the zinc within the MMPs can modulate enzymatic activity.

In view of the evidence for the potential interaction between heavy metals and pathways of regulation of MMP expression and activity, we undertook to examine the role these enzymes may play in the tissue response to heavy metal poisoning. We have begun to isolate cDNA clones for species-specific MMPs from the dogfish shark rectal gland using degenerate oligonucleotide primers directed against highly conserved regions shared among the members of the MMP family. We plan to use these cDNA probes to analyze the pattern of mRNA expression of the MMPs in a range of normal tissues. Studies will then be undertaken to determine the activity of these genes in response to exposure to heavy metals (mercury, cadmium, nickel) in cultured shark rectal gland cells and in isolated perfused shark rectal glands.

Cloning of the species-specific MMPs was initiated by exploiting the high degree of conservation between two specific regions shared by the whole family of enzymes, namely the "cysteine switch" region and the zinc binding site. These sequences are highly conserved evolutionarily, and have been documented to show homology between mammals and animals as distantly related as the sea urchin (Lepage T., Gache C., EMBO 9: 3003, 1990). An aliquot of the dogfish shark rectal gland library was heated to 95° C to disrupt the phage coat and inactivate endogenous proteases. Subsequently, primers, nucleotides, buffer, and Taq polymerase were added and a low-stringency PCR performed for thirty cycles. Because gelatinases contain a unique fibronectin binding domain absent from collagenases, these two groups of enzymes give a characteristic pattern on PCR,

yielding two species of approximately 800bp and 300bp respectively.

PCR was undertaken over a range of temperature and Mg concentrations, and PCR products of the predicted size (300bp and 800bp) were seen. The PCR products were directly cloned into the TA vector system (Stratagene), and restriction digest confirmed inserts of 300 and 800 bp. These plasmids are currently being sequenced to confirm their identity as MMPs. DNA inserts with intact open reading frames and homology to MMPs will be used to screen the parent library to allow us to obtain full-length clones of the cDNAs. These will be further analyzed for their homology to known MMPs from higher species. Although fragments of both predicted sizes (300bp and 800bp) have been obtained, the identity of these fragments must be established by sequencing, as it is quite common to obtain unrelated fragments of coincidentally expected size by low stringency PCR.

It is our hypothesis that study of the pattern of MMP expression in response to heavy metals could provide important insights into the pathogenesis of clinical syndromes associated with toxic metal exposure. Furthermore, it is possible that relevant studies of metalloproteinase expression in response to heavy metals could provide a general model for tissue injury applicable to a wide range of lesions. There is intense interest in the pharmaceutical industry in developing specific inhibitors to metalloproteinases. If it could be established that the pathogenesis of tissue injury involves overexpression of MMPs, this could broaden the potential applicability of such agents.

We are currently sequencing the DNA inserts cloned from the PCR products last summer. Once an insert with specific MMP sequences is identified, this will be used to clone a cDNA clone from the rectal gland library. At the same time, the insert can be used directly as a probe to examine the mRNA expression of the identified gene in shark rectal glands isolated from animals perfused with heavy metals. These results will be correlated with examination of histologic changes induced by heavy metal perfusion.

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