ROLE OF METALLOPROTEASES IN MEDIATING HEAVY METAL TOXICITY IN *SQUALUS ACANTHIUS*

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The matrix metalloproteases (MMPs) are matrix modifying enzymes which play diverse roles in tissue remodeling associated with differentiation, wound healing, and the inflammatory response, as well as in tissue invasion and metastasis (Matrisian, *Trends in Genetics* 6: 121-125, 1990; Stetler-Stevenson et al, *Ann Rev Cell Biol* 9: 541-73, 1993; Woessner, *FASEB J.* 5: 2145, 1991). Over the past two summers, we have sought to characterize the role of metalloproteases in the tissue injury associated with heavy metal poisoning. This summer's work proceeded in two directions: 1) continued efforts to isolate an MMP cDNA clone from a *Squalus acanthius* rectal gland library, and 2) efforts to purify metalloprotease protein from tissue.

The activity of MMPs is regulated at several levels. Transcriptional activity of the MMP genes is tightly regulated, and can be postulated to be potentially modulated by heavy metals. 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. Second, the MMPs are synthesized as inactive zymogens which must be cleaved to generate active enzymes. Collagenases are activated *in vitro* by organomercurials, indicating that interaction of other metal ions with the zinc within the MMPs may modulate enzymatic activity (Mookhtiar KA et al. *Analytical Biochemistry* 158: 322-333, 1986). Third, MMP activity is further modified by tissue inhibitors of metalloproteases (TIMPs).

In view of the evidence for the potential interaction between heavy metals and pathways of regulation of MMP expression and activity, we continued our attempts to obtain 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. All metalloproteases contain a highly conserved histidine-rich sequence which represents the binding site for zinc; zinc binding is necessary for enzyme function, which is inhibited by chelating agents. The latency of the MMPs depends on another highly conserved sequence motif, termed the "cysteine switch," which is thought to form a complex with the zinc atom.

The zinc binding site and the cysteine switch region sequences are highly conserved both between MMPs and in evolution. These conserved regions were used to generate oligonucleotide primers with which to amplify shark-specific MMP sequences from the rectal gland cDNA library. Over the past two summers we have successfully amplified what appear to be the appropriate size fragments from the cDNA library; however, technical problems with cross-contamination with human sequences, and reiterated homologous sequences which have resulted in amplification with the same oligonucleotide at both ends, have prevented successful isolation of an appropriate cDNA. We are currently sequencing further subcloned fragments in an attempt to identify shark-specific MMP sequences.

In the meantime, during the summer we performed mercury and cadmium infusions of isolated rectal glands with the assistance of Dr. John Forrest's laboratory. We have frozen tissue for RNA isolation for further studies once the cDNA probe is available. We plan to analyze the pattern of mRNA expression of the MMPs in

normal tissues and in response to exposure to heavy metals in these isolated perfused shark rectal glands.

Additional experiments were directed at characterizing metalloprotease activity in fresh tissue. A fluorescent substrate specific for the MMP matrilysin was used to assay tissue homogenates of dogfish heart, liver, muscle, rectal gland, and testis for protease activity. Liver, rectal gland, and testis were all found to have significant proteolytic activity which was inhibited by the metalloprotease inhibitor *o*-phenanthroline. Further studies are being directed at purifying isolated enzyme from liver homogenates. If successful, tryptic digests will yield fragments for peptide sequencing; this can be used to make oligonucleotide primers for direct screening of the cDNA library if the PCR-based approach continues to be unsuccessful.

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, studies of metalloprotease expression in response to heavy metals may 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 were established that the pathogenesis of tissue injury involves overexpression of MMPs, this could broaden the potential applicability of such agents.

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