Matrix Metalloproteinases Expression during Limb Regeneration

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Abstract

Axolotl (regeneration-competent) is one of the unique vertebrates which can regenerate missing organs such as limbs, jaws, spinal cord, and tail anytime during their life cycle. There also exists a recessive mutant of axolotl which has a phenotype called short toes (s/s, regeneration-deficient). The s/s mutant can regenerate its tail and spinal cord but cannot maintain the growth of the limb blastema, which results in the failure of limb regeneration. Remodeling of extracellular matrix (ECM) during early blastema formation, also known as histolysis, leads to the release of stem cells and activation of various growth factors. Therefore, histolysis is considered to be a crucial step in regenerating the exact replica of missing limbs in axolotls. Matrix metalloproteinases (MMPs) are zinc dependent endopeptidase that have been suggested to play roles in histolysis. However, it still remains unclear if histolysis is different in limb regeneration between regeneration competent and deficient animals. In this study, we analyzed the expression patterns of MMPs and the tissue inhibitors of the MMPs (TIMPs) in axolotl and s/s utilizing MMP arrays (RayBiotech, Inc., Norcross, GA), zymography and western blots. The cut-off limbs of the axolotls and s/s were used as controls. The animals were allowed to regenerate and the blastema was collected at three stages: epidermis closure (EC), dedifferentiation (DD), and early bud (EB). The total proteins were extracted from all the samples. 20 µg of protein was used to perform MMP arrays according to manufacturer's protocol. They detected MMP-1, -2, -3, -8, -9, -10, and -13, as well as TIMP-1, -2 and -4 in the controls, EC, DD and EB samples from axolotl and s/s. Gelatin zymograghy with 20 µg of protein confirmed that MMP-2 and -9 were expressed at all the same time points in the axolotl and s/s samples. The expression patterns of MMP-9 were similar in the axolotl and s/s until the DD stage. While later in the EB stage, the axolotl showed a decrease in MMP-9 expression and s/s showed increased expression. Western blots were performed with 40 µg of protein using MMP-2 and -9 antibodies, and confirmed the zymography results. These results suggested that the expression patterns of the MMPs, especially MMP-9, are different in regeneration competent and deficient animals. One of the keys for a healthy blastema formation, which can multiply and later repattern into the missing limb, might be the release of the critical amount of MMP at the right time. This study was supported by an IUSD start-up grant to F. Song and a grant from W. M. Keck Foundation to D. L. Stocum.