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REGENERATION IN ZEBRAFISH (DANIO RERIO) FINS: PATTERN OF EXPRESSION OF MINERALIZATION–RELATED GLA PROTEINS

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Teleost fishes have the exceptional ability to largely regenerate severed appendages comprising several different tissues. Fin regeneration starts through the formation of heterogeneous mesenchyma-like cells, named blastema, and located between stump tissues and the wounded epidermis. This event, denominated epimorphic regeneration, comprises strict growth control and cell reprogramming leading to faithful restoration of the lost parts. Matrix Gla Protein (Mgp) and Bone Gla Protein (Bgp, osteocalcin) are small extracellular matrix Gla proteins, members of the vitamin K-dependent (VKD) family. These proteins are considered to be related to bone formation and mineralization, and more recently, to vascular calcification. Bgp is associated with the extracellular matrix of mineralized tissues while Mgp accumulates mainly in the extracellular matrix of calcified cartilage and in the vascular system. The typical teleost caudal fin, such as the one in zebrafish, is composed of multiple fin rays with a bony part named lepidotrichium, so it is of great relevance to determine Mgp and Bgp patterns during regeneration events.
In this work our objective was the identification of expression patterns of mgp and bgp during regeneration of adult zebrafish caudal fins, particularly in fin rays. In situ hybridisation was used to identify specific sites of expression for each of these genes while the histological markers alizarin red and alcian blue allowed us to detect both calcium deposition and cartilage formation in the regenerating fin. The results obtained formed the basis for a more extended study on the effects of various aquatic pollutants on fin regeneration.

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Osteocalcin (*Oc*) and Matrix Gla protein (*Mgp*) are members of a small family of proteins that contains γ-carboxylated glutamate (Gla) residues resulting from a vitamin K dependent post-translational modification. Gla residues are thought to mediate the binding of mineral ions to these proteins, which affect tissue calcification. While *Mgp* is mainly located in cartilage, osteocalcin accumulation is restricted to bone. The presence of these proteins was reported in unspecific sites either associated with pathogenic conditions or induced in laboratory. In normal fish, osteocalcin and *Mgp* were predominantly found in the same sites of accumulation as in tetrapods but nothing is known concerning the expression of these proteins in malformed fish. Using immunohistochemical and histological techniques, we analysed the presence of osteocalcin and *Mgp* in vertebra of deformed and normal fish of different species, and evaluated the possibility of these proteins being used as molecular markers for the presence of skeletal deformations in fish. Levels of gene expression were also compared in normal and deformed vertebrae by quantitative real time PCR. While Mgp showed no significant differences, osteocalcin was found to accumulate in non mineralized notochord cells within vertebral bodies (chordoblasts and chordocytes), but only in lordotic fish. This discovery may contribute to better understand the mechanisms of skeletal deformations, one of the most severe and costly problems of Mediterranean aquaculture.

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