RECOMBINANT HUMAN BONE MORPHOGENETIC PROTEIN-2 (BMP-2) STIMULATES A DERMAL OSTEOGENIC WOUND RESPONSE IN THE SKATE BUT NOT IN THE SHARK: AN EARLY VERTEBRATE CLUE TO THE FORMATION OF A DERMAL EXOSKELETON IN CHILDREN WITH PROGRESSIVE OSSEOUS HETEROPLASIA

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The bone morphogenetic proteins (BMPs) are a family of potent morphogens in the transforming growth factor-beta superfamily of peptides, and induce endochondral osteogenesis and fracture healing at endoskeleton sites in the higher vertebrates. These substances act by diffusion in a concentration-dependent manner to specify the fate of cells during embryogenesis and skeletal regeneration. BMPs are also unique in their ability to induce the complete cellular program of endochondral osteogenesis at heterotopic sites in higher vertebrates. Several of these molecules (BMP 2 & 4) are involved in the specification of the dorsal-ventral body plan of all animals, in the formation and regeneration of the skeletal and hematopoietic systems in vertebrates, and in the induction of mesoderm throughout the chordate phylum.

We undertook this experiment in order to determine the skeletogenic response of the deep connective tissue in cartilaginous fish to implanted recombinant human BMP (rhBMP). We hypothesized that rhBMP would induce ectopic chondrogenesis in the deep musculature of the cartilaginous fish and would induce endochondral osteogenesis in the deep musculature of a bony fish.

Type I collagen sponges (Helistat sponge; Collatec Inc., Plainsboro, N.J.) were saturated with 30 micrograms of recombinant human BMP-2 (rhBMP-2) @ 100 micrograms per ml; Genetics Institute, Cambridge, Mass.), and implanted in a subcutaneous site of the dorsal aspect of two dogfish sharks (*Squalus acanthias*), two skates *(Raja erinacea)*, and a teleost (sculpin) (*Myoxocephalus octodecimspinosus*). Sponges saturated with diluent but without rhBMP-2 were implanted in a similar location in another site on the opposite side of the midline in each animal. Wounds were closed with a silk suture. The fish were returned to a holding tank supplied with fresh flowing sea water at an ambient water temperature of 14 - 16 degrees centigrade.

Eighteen days later, the fish were sacrificed, the wound sites excised, and the tissue samples fixed in 10 percent phosphate buffered formalin brought to elasmobranch isotonicity with sodium chloride (10 percent buffered formalin, 0.1 molar phosphate buffer, 360 millimolar sodium chloride, pH 7.2). The sculpin died on day-17, but the tissue was harvested at day-18.

Tissue specimens were processed by standard histological procedures for light microscopy. Specimens were imbedded in paraffin, sectioned at 2 microns, stained with hematoxylin and eosin or von Kossa reagent and viewed under light microscopy.

Surgical implantation of a collagen sponge in the shark caused myxoid degeneration in the dermis and in the deep skeletal muscle. There was an extremely mild inflammatory reaction with scant lymphocytes and neutrophils. The wound response following implantation of a BMP-2 soaked collagen sponge was identical to that of the control sponge in both the superficial and deep tissues. There was no evidence of either cartilage or bone formation in the muscle, dermis, or epidermis.

The wound response in the skate was dramatically different from that in the shark. In the control site, small primitive acellular bone plates formed at the wound site in the dermis. These islands of bone were acellular internally but rimmed with cuboidal cells that were secreting matrix. These plates were also mineralized, demonstrated by the von Kossa stain. There was no bone or cartilage in the deeper muscle, but degenerating muscle fibers and a very mild inflammatory response were observed. In the site implanted with a BMP-2 soaked collagen sponge, a dramatic enhancement of primitive acellular bone was seen in the dermis at the wound site and around the wound site, but no bone or cartilage formed in the deep muscle.

The wound response in the teleost (sculpin) was similar to that of the skate and showed abundant acellular bone in the dermis. The response to the BMP-2 soaked collagen sponge was similar in the sculpin and in the skate and revealed a dramatic enhancement of intramembranous-type ossification at the surgical site. There was no cartilage or bone in the deeper muscle tissue.

The major findings of our study were: 1) that a deep surgical wound leads to intramembranous heterotopic ossification in the dermis of a skate, but not in a shark, and 2) the dermal osteogenesis was greatly enhanced in the presence of recombinant human BMP-2. The dermal osteogenic wound response in the skate and its enhancement with rhBMP-2 were entirely unexpected, while the expected wound response in the deeper tissue did not occur.

In a rare genetic disorder, progressive osseous heteroplasia (POH), spontaneous intramembranous ossification occurs in the skin and invades the deep muscular tissues in a mosaic distribution. The molecular pathogenesis of POH is unknown. The histological appearance of intramembranous heterotopic ossification in the dermis of the skate was similar to that seen in children who have POH. Unlike in POH, however, there was neither a chondrogenic nor an osteogenic response in the deeper muscle tissue of the skate.

In a meticulous examination of the development and evolutionary origins of vertebrate skeletogenic tissues, Smith and Hall (Biol. Rev., 65: 277-373, 1990) contend that "the first vertebrate skeleton was exoskeletal and was derived from neural crest". The authors suggest that the neural crest is the source of the vertebrate dermal exoskeleton. The BMPs have recently been shown to be lineage determination signals in neural crest development and differentiation.

Our preliminary findings raise many provocative questions:

1) What are the molecular and the epigenetic factors that determine the development of cartilage or bone in the primitive elasmobranchs?

2) What is the molecular basis for the difference in the wounding response in the shark and the skate? What are the molecular factors and pathways that either promote a dermal osteogenic wounding response in the skate or prohibit it in the shark?

3) Do sharks and skates have the full complement of functional bone morphogenetic protein genes that exists in higher vertebrates who have bony endoskeletons?

4) Are BMPs expressed normally in the skin of the cartilaginous and bony fish? Is there a difference in BMP expression in these animals? Are BMPs and BMP receptors expressed in the skin of mammals and if so, in what cells?

5) What is the tissue origin of the osteoblasts involved in intramembranous dermal osteogenesis in the skate? Are these cells of neural crest origin? Is the origin of the dermal osteoblast different in the cartilaginous and bony fish?

6) What is the germ-line origin of the dermal exoskeleton in children who have POH? Are the cells of neural crest origin?

7) Are there naturally occurring BMP-antagonists produced in this shark that inhibit the production or action of dermal osteogenesis, and if so, what is their effect on dermal osteogenesis in the skate and in the bony fish?

In summary, our preliminary data suggest that the epidermal-dermal interaction that occurs as a result of a surgical wound in the skate (but not in the shark) leads to dermal osteogenesis which is markedly enhanced in the presence of rhBMP-2. This remarkable production and enhancement of dermal osteogenesis in an elasmobranch is strikingly similar to the intramembranous heterotopic ossification that occurs in the dermis of children who have the rare heritable disorder progressive osseous heteroplasia. These bone morphogenetic protein clues from the cartilaginous fish have implications for understanding the pathophysiology of a disabling dermatologic and musculoskeletal condition in man.