PEPROTECH®
OUR SUPPORT, YOUR DISCOVERY

FOCUS

Bone and Bone Morphogenetic Proteins

The rigid bone, the flexible cartilage, and the elastic tendon exemplify the remarkable capacity of connective tissue to assume a wide range of physical states. Connective tissue originates from the mesoderm of the embryo, and develops from fibroblastic cells of the mesenchyme. It supports and binds other parts of the body, and dictates the morphologic characteristics of the organism. The tissue is firm, consisting of a large number of cells, mostly histocytes, caged in a compact network of extracellular matrix. The matrix is composed primarily of high molecular weight proteins such as collagen, elastin and reticullin. Collagen, which comprises about one third of the total protein in mammalian organisms, is the fundamental constituent of most connective tissues including the dermis, tendons, ligaments, deep fascia, bone and cartilage. It is a strong fibrous substance, which exists in several distinct types. These collagen types differ slightly from one another in their amino acid sequence, but they all share a common quaternary structure composed of three polypeptide α-chains arranged in a triple helical conformation. Bone matrix, known as osteoid, is made predominantly (90-95%) of type I collagen, while cartilage matrix contains mainly type II collagen. The collagenous matrix in cartilage is coated with tree-like branches of proteoglycan molecules, which render the tissue flexible. The rigidity of bone, on the other hand, is due to mineralization of the matrix, which occurs shortly after the osteoid is inundated with mature bone cells. Mineralization of the bone involves rapid accumulation of amorphous calcium phosphate followed by gradual conversion of the mineral into crystals of hydroxyapatite.

The three major cell types found in bone are osteoblasts, osteocytes and osteoclasts. Osteoblasts, or bone forming cells, originate from pluripotent mesenchymal stem cells that can also differentiate into muscle, fat, and blood cells. Mature osteoblasts synthesize and extrude the necessary proteins for bone construction, including type I collagen, osteocalcin, osteonectin and alkaline phosphatase. Osteocalcin is a 6 kDa calcium binding protein, capable of capturing calcium from the circulation. Osteonectin is a 32 kDa protein that serves as glue between the collagenous matrix and hydroxyapatite. Alkaline phosphatase is an enzyme that facilitates proper bone mineralization. Once entrapped within the mineralized bone, the osteoblast loses its synthetic activity and becomes a resting osteocyte. The metabolic activity of osteocytes helps to maintain the calcified bone as a living tissue. The osteoclast is a giant multinucleated cell derived from fusion of several precursor cells of the monocyte/macrophage lineage. The primary function of osteoclasts is bone resorption, a process that involves both extraction of calcium and destruction of matrix. Normally, bone resorption occurs concomitantly with new bone formation. The coupling of these processes, known as bone remodeling, enables continuous renewal of bone mass throughout the life of the organism. It also provides bone with the capacity to heal itself from breaks and cracks.

Ossification, or the conversion of tissue to bone, involves destruction and removal of tissue and formation of bone in the space formally occupied by it. It normally occurs in either membranous fibrous tissue or in cartilage, as in the formation of the cranial and long bones, respectively. It is a multi-step process that includes the following cascade of events: (I) attraction and attachment of mesenchymal stem cells (MSCs) to the collagenous matrix, (II) proliferation and differentiation of MSCs into cartilage and bone cells, (III) destruction of the original matrix coupled with formation of new bone matrix, (IV) vascularization and mineralization of the bone, (V) bone remodeling associated with proliferation and differentiation of hematopoietic stem cells. The end result is the replacement of the original tissue with a new bone, complete with functional marrow.

A comprehensive characterization of ectopic ossification was described in 1965 by Urist, who discovered that acellular, devitalized and demineralized bone matrix (DBM) could induce cartilage and bone formation when implanted under the skin or into the muscle of adult rodents (1). The osteoinductivity, or bone inducing activity of DBM was later attributed to a proteinacious component that received the name bone morphogenetic protein (BMP) (2). Further studies indicated that although BMP could be solubilized and extracted from the matrix, an insoluble osteoconductive carrier such as collagen sponge or synthetic hydroxyapatite was critical for manifestation of its in-vivo activity (3,4). Protein purification followed by amino acid sequencing of several BMP-derived tryptic fragments, led to the cloning and expression of the first group of BMPs (5-7). Today, these proteins constitute a family of more than 30 known members, 15 of which are

Bone and Bone Morphogenetic Proteins continued...

of mammalian origin. Members of the BMP family are also known by other names such as osteogenin (BMP-3), osteogenic protein-1 or OP-1 (BMP-7), and cartilage-derived morphogenetic protein-1 or CDMP-1 (BMP-14). As implied by their name, BMPs initiate, promote, and regulate bone development, growth, remodeling and repair (8). However, it is now clear that in addition to their roles in bone and cartilage morphogenesis, BMPs are also involved in prenatal development and postnatal growth of eye, heart, lung, kidney, skin, and other tissues.

The amino acid sequences of homologous BMPs from different mammalian species are highly conserved, some are even identical in several species e.g. mouse, rat, and human BMP-2, and bovine and human BMP-10 and -11. With the exception of BMP-1, which mistakenly was called BMP, but is in fact a type I procollagen C-proteinase (9), the BMPs belong to the TGF-β superfamily of structurally related signaling proteins. Members of this superfamily are widely represented throughout the animal kingdom, and have been implicated in a variety of developmental processes. Proteins of the TGF-β superfamily are disulfide-linked dimers composed of two 12-15 kDa polypeptide chains (monomers). Each of these monomers contains seven absolutely conserved cysteine residues. These proteins exist mostly as homodimers but some are heterodimers e.g. monomers of BMP-2 and -7 can associate together. Their mature and active form, obtained by proteolytic cleavage of biologically inactive precursors, corresponds to approximately the C-terminal one third of the precursor molecule. The crystal structure of some of these proteins, e.g. BMP-2 and TGF-β2, revealed a characteristic structural motif consisting of a cysteine-knot with two finger-like double-stranded sheets (10).

The potential clinical uses of BMPs in treating bone and cartilage defects have stimulated extensive research. During the past decade, the osteogenic activities of these proteins have been tested in combination with a variety of osteoconductive carriers both in human and animal models (for review see ref. 11). These studies have demonstrated the efficacy of some of these proteins, for example BMP-2 and BMP-7, in bone-repair experiments. Recently, a bone-graft substitute, called OP-1™ Implant, made of recombinant human BMP-7 combined with bovine bone-derived collagen, has been approved by the FDA as a device for treating fractured bones that do not heal after a normal period of time. Additional applications of BMPs, alone and in combination with one another, in orthopedic and dental reconstructive surgeries continue to be a subject of intense investigation. The availability of new recombinant BMPs (12) should facilitate further research aimed towards the development of improved tools for treating bone and cartilage defects.

References

(1) Urist M.R., Science **150**, 893-899 (1965)
(2) Urist M.R., et. al., Proc. Natl. Acad. Sci. USA **76,** 1828-1832 (1979)
(3) Sampath, T.K., and Reddi, A.H. Proc. Natl. Acad. Sci. USA **78,** 7599-7603 (1981)
(4) Ripamonti U., et al., Matrix **12**, 202-212 (1992)
(5) Wang E.A. et al., Proc. Natl. Acad. Sci. USA **85**, 9484-9488 (1988)
(6) Wozhey J.M., et al., Science **242,** 1528-1534 (1988)
(7) Ozkaynak E., et al., EMBO J. **9,** 2085-2093 (1990)
(8) Lens, S.M. et al., Semin. Immunol. **10**, 491-499 (1998)
(9) Amano, S. et al., J. Biol. Chem. **30,** 22728-22735 (2000)
(10) Scheufler, C. et al., J. Mol. Biol. **287**, 103-115 (1999)
(11) Groeheveld, E.H.J and Burger E.H., Europ. J. of Endocrinology 142, 9-21 (2000).
(12) See new product list on this page.

Relevant products available from PeproTech

Human Adiponectin	Catalog #: 450-24
Murine Adiponectin	Catalog #: 315-26
Human gAcrp30/Adipolean	Catalog #: 450-21
Murine gAcrp30	Catalog #: 450-27
Human gAcrp30/Adipolean Variant	Catalog #: 450-20
Human Leptin	Catalog #: 300-27
Murine Leptin	Catalog #: 450-31
Human Nesfatin-1	Catalog #: 300-67
Human Resistin	Catalog #: 450-19
Murine Resistin	Catalog #: 450-28
Human TNF-α	Catalog #: 300-01A
Murine TNF-α	Catalog #: 315-01A
Rat TNF-α	Catalog #: 400-14