3.1.1.1 Identification of Human BMP Genes

In 1965, Marshal Urist showed that new bone formation could be induced using demineralized bone matrix (DBM). This observation suggests that a protein, or a group of proteins, present in bone has the capacity to repair and regenerate bone. This class of proteins was named bone morphogenetic protein (BMP) and his work prompted studies in numerous laboratories that led to the development of osteoinductive bone graft substitutes. These proteins were isolated in sufficient quantity and purity to provide amino acid sequence data thus allowing cDNA clone isolation and characterization. OP-1 was one of the first of the BMP genes identified and expressed as a recombinant protein. Recombinant human OP-1 is now produced by the Chinese hamster ovary (CHO) cell line, DXB11, and purified from the conditioned media. This cell line was engineered for expression of the OP-1 gene, under control of the cytomegalovirus major immediate early (CMV-MIE) promoter.

The BMP family of proteins is the largest subgroup of the transforming growth factor-β (TGF-β) superfamily. TGF-βs act as signaling molecules in tissue and organ development and have a wide range of functions in connective tissue, brain, kidney, and muscle. Proteins from the superfamily are classified into subgroups based on sequence conservation within the C-terminal 7-cysteine domain (also called the TGF-β domain). In addition to BMPs, other families include the activin/inhibin and TGF-β subgroups. Currently, there are approximately 30 members of the BMP family.

BMPs are distinguished from other bioactive factors by a mechanism of action that induces a differentiated phenotype. BMPs are differentiation factors, causing pluripotent mesenchymal cells to terminally differentiate (mature) into a number of specialized cell types including osteoblasts, chondrocytes, myoblasts, fibroblasts, and adipocytes. In contrast, other growth factors such as epidermal-derived growth factor (EGF) cause cells to divide rapidly leading to a dedifferentiated phenotype often observed in transformed cells. BMPs, including OP-1, frequently inhibit proliferation in tumor cells by promoting differentiation.

The structure of OP-1 is very well characterized. It is synthesized in the CHO cell as a 431 amino acid precursor protein that is subsequently processed during secretion to a 263 amino acid prodomain and a 139 amino acid mature domain. These two domains remain noncovalently attached following secretion (Figure 2-1).
During the purification process, the pro and mature domains are separated and the active form of the protein is purified. Mature OP-1 is a dimer of two 139 amino acid chains connected by a disulfide bond with an approximate molecular weight of 36 kDa. Each monomer contains three N-linked glycosylation sites with the major site of glycosylation at Asn 80.

The sequence of the mature protein is highly conserved among species. In the TGF-β domain there is only one amino acid difference between mouse and human OP-1, and in the entire mature protein only 3 differences occur. Consequently, comparisons of experiments from different species and extrapolation of results across species should have relevance in humans.

The biologic effects of OP-1 and other BMPs are transduced by the formation of a heteromeric receptor complex on the cell surface. The complex consists of two Type I and two Type II receptor proteins, which are transmembrane serine/threonine kinases. Three Type I receptors (ActR-I, BMPR-IA, and BMPR-IB) and three Type II receptors (ActR-II, ActR-IIB, and BMPR-II) have been shown to associate with one or more members of the BMP family. BMP family members bind to the various Type I and Type II receptors with differing affinities. Natural inhibitor proteins such as noggin, chordin, and DAN bind to BMPs and inhibit their interactions with receptors. Furthermore, the binding of BMPs to extracellular matrix components such as collagen and heparin sulfate influences their abilities to interact with receptors. The intracellular signaling pathways that are induced by the serine/threonine kinase receptors involve a family of signaling molecules called Smad proteins. They can be divided into three subclasses: R-Smads (receptor-activated Smads), Co-Smads (common partner Smads), and anti-Smads (inhibitory Smads). Following BMP-induced phosphorylation, R-Smads dissociate from the receptor, bind to Co-Smads, and enter the nucleus. Inside the nucleus, heteromeric complexes of Smads regulate transcription by utilizing DNA-binding proteins to target specific genes.
Although BMPs were originally discovered in bone, they are expressed in most tissues of the human body.\[xiv\] During embryogenesis, the BMPs serve as important inductive signals for tissue development and have been shown to play a pivotal role in development of the musculoskeletal system, nervous system, heart, kidney, skin, eyes, and teeth. After birth, the BMPs are involved in tissue repair and regeneration. In localization studies in adult mice, OP-1 mRNA is found in the kidney, bladder, adrenal tissue, brain, and calvaria.\[x\] In mouse embryos, OP-1 mRNA is found in multiple organs at levels that vary depending upon the time after conception. OP-1 gene knockout studies have proven the importance of OP-1 during embryogenesis.\[xv\] Mice lacking the OP-1 gene display defects in the developing kidney and eye, and appear polydactyl; as expected, knockouts do not live past birth due to the lack of functioning kidneys.

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