บทนำ

Human articular cartilage is an avascular and anerval tissue composed of a highly organized and specialized acellular component, the extracellular matrix, and a spare cellular component, the chondrocytes. In adult organism, it shows a slow tissue turnover and remodeling. Chondrocytes are surrounded by an abundant extracellular matrix (ECM) which composed of types of collagen (mainly collagen type II, IX and XI), proteoglycans (mainly aggrecan) and various types of glycoproteins such as fibronectin. They have a characteristic by low rate of cell division in the articular cartilage and their ability to expand the extracellular matrix containing the collagenous proteins and proteoglycans. Cartilage tissue engineering using chondrocytes as initial precursor requires the expansion of cell numbers on monolayer culture. The acquisition of chondrocytes for autologous transplantation is faced a major problem involving the losing their characteristic phenotype, collagen type II expression, fibroblastic appearance and proliferation. Dedifferentiated chondrocytes are found rapidly during the *in vitro* cultivation. Alternatively, the use of pluripotent or multipotent stem cells in place of committed tissue-specific cells represents an exciting new approach.

Mesenchymal stem cells (MSCs) are adult stem cells that constitute a variety of adult tissues. MSCs maintain the self-renewal ability and the capability to differentiate to various connective tissues including cartilage and bone. *In vivo* implantation of these cells at orthotopic sites will also yield tissues in these lineages. The main source of MSCs is the bone marrow. MSCs are also located in other tissues like: adipose tissue, peripheral blood, umbilical cord blood, liver and fetal tissues. The induction of chondrogenesis in MSCs depends on the coordinated activities of many factors, including parameters such as cell density, cell adhesion, and growth factors. Bone Morphogenetic Proteins (BMPs) are the key regulatory factors in chondrogenic and osteogenic differentiation, and also function in repair and remodeling of the adult skeletal system. BMPs exert diverse biological process ranging from early embryonic tissue patterning to postnatal tissue homeostasis. BMPs have been involved in the regulation of cell proliferation, survival, differentiation, apoptosis and stem cell properties. BMPs stimulate osteogenic differentiation in mesenchymal progenitor cells as well as increase stem cell numbers. BMPs are the multi-functional growth factors belonging to the TGF-β superfamily. Members of BMP family bind to two distinct type II and type I serine/threonine kinase receptors. BMP binding leads to dimerization of receptors prior to phosphorylation and signaling through the Smad pathway. In recent years experimental evidence accumulated that BMPs are the potent anabolic factors for development of articular cartilages and play their roles for the maintenance adult articular cartilage. The overlapping biological activities of

BMPs raise the possibility that each BMP molecule possesses distinct roles at determined time points in restricted sites during organogenesis or pattern formation. Among BMPs have been identified, BMP-2 has significant importance in chondrogenesis, bone development and the proteoglycan biosynthesis. Thus, BMP-2 is suggested as a promising candidate in cartilage tissue engineering of joint defects. The more information about the action of human BMP-2 on development and its role on chondrocyte is needed to be fulfilled. Therefore, it leads to interest for investigation of the human BMP-2 function on development and proliferation of bone and chondrocyte.

วัตถุประสงค์ของโครงการ

เพื่อผลิตรีคอมพ์บิแนนท์โปรตีนสำหรับ Bone Morphogenetic Protein-2 ของมนุษย์ โดยทำการโคลนยีนที่แยกได้จากเซลล์กระดูกระยะตัวอ่อน ศึกษาการแสดงออกของยีน และแยกบริสุทธิ์โปรตีนโดยอาศัยเซลล์เจ้าบ้านแบคทีเรีย