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Review

Metaplasia of Chondrocytes into Osteoblasts

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Hypertrophic chondrocytes, commonly considered as terminal cells responsible for apoptotic elimination in endochondral osteogenesis, have the potential to switch their metabolic role and enter osteoblastic differentiation, based on histochemical, immunohistochemical, biochemical and cytological analysis.During endochondral osteogenesis, some osteocytes are derived from hypertrophic chondrocytes. Also non-hypertrophic chondrocytes are able to transform into osteogenic cells, and the bone thus formed is termed "transchondroid bone". In this review a summary and discussion of reports on chondrocyte transdifferentiation is given.

Key words: Hypertrophic chondrocytes, transdifferentiation, chondroid bone.

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Generally, two osteogenic models are recognized: direct bone formation by differentiation of mesenchymal cells into osteoblasts (intramembranous osteogenesis) and indirect, so called endochondral osteogenesis. In the latter case the chondrocytes proliferate, mature and differentiate into hypertrophic chondrocytes, surrounded by mineralising cartilaginous matrix, which undergo apoptosis and die. According to ADAMS and SHAPIRO (2002) the signal for hypertrophic chondrocyte apoptosis emerges from the modified, mineralized cartilaginous matrix. Thus in endochondral osteogenesis, hyaline cartilage serves merely as a scaffold for osteogenesis, led by cartilage- invading mesenchymal cells. These cells, delivered by vascular buds, occupy the vacant chondrocytic lacunae and deposit bone on the surface of the cartilaginous matrix eroded by chondroclasts. Gradually, areas of hypertrophic cartilage are replaced by bone produced by mesenchyme- derived osteoblasts.

Although it is accepted that hypertrophic chondrocytes degenerate (BRIGHTON *et al*. 1973) or undergo apoptosis (LEWINSON & SILBERMAN 2002; HATORI *et al*. 1995; GERSTENFELD & SHAPIRO 1996; ADAMS & SHAPIRO 2002), some authors have claimed that hypertrophic chondrocytes survive, sustained by blood vessels invading chondrocytic lacunae and are transformed into osteoblasts (HOOLTROP 1972; SHIMOMURA *et al*. 1975; GERSTENFELD & SHAPIRO 1996; SCAMMELL & ROACH 1996).

In embryonic life, flat bones (e.g. skull bones) are formed by intramembranous osteogenesis. This type of osteogenesis can lead to bone formation after birth anywhere that it is not preceded by cartilage formation, for eg. in extraskeletal osteogenesis, or during bone fracture healing. In embryonic life, endochondral osteogenesis determines the histogenesis of long bones, whilst postnatally, it takes place in the epiphyseal plates, until maturity. It is responsible for bone length, as well as in fracture healing, where bone regeneration is a mixture of both osteogenic models.

In both types of osteogenesis cartilage and bone cells are derived from mesenchymal cells.

Experiments conducted in the last three decades have revealed a new mode of osteogenesis, which relies on the direct transformation of cartilaginous cells – chondrocytes – into bone-forming cells –

osteoblasts. This peculiar type of osteogenesis is named "transchondroid ossification" (YASUI *et al*. 1997; KAWAKAMI *et al*. 2001). The process of transformation of one differentiated cell into another type of differentiated cell, for example chondrocytes into osteocytes, is called transdifferentiation, replacing the former name – metaplasia.

Transdifferentiation – manifestation of a cell's plasticity, can be observed both *in vivo* and *in vitro*. Overexpression of transforming growth factor in endothelial cells of arterial vessels induces transdifferentiation of smooth muscles into cells revealing a chondrocytic phenotype (SCHULICK *et al*. 1998; BOBRYSHEV 2005). Human prostate fibroblasts under the influence of this cytokine convert into cells having myocyte characteristics (so called myofibroblasts) (UNTERGASSER *et al*. 2005). Expression of Runx2 transcription factor 1 in skeletal myogenic cells has induced their transdifferentiation into a mineralizing osteogenic lineage (GERBASH *et al*. 2004). *In vitro*, stromal bone marrow cells, in contact with glial Schwann cells can differentiate into nerve cells (ZURITA *et al*. 2005), whilst Schwann cells, by the influence of transforming growth factor beta-1, can redifferentiate into myofibroblasts (REAL *et al*. 2005). Differentiated cells, derived from mesenchymal stem cells, or from murine embryonal stem cells (chondrogeneic, osteoblastic and adipocytic cells), were re-differentiated into another cell type (e.g adipocytic into osteoblastic or chondroblastic, and vice versa) in response to modified culture medium. Such transdifferentiation was preceded by extensive proliferation of de-differentiated cells (HEERMEIER *et al*. 1994; HEGERT *et al*. 2002; SONG & TUAN 2004).

In chondrosarcoma, evidence for transdifferentiation of hypertrophic chondrocytes into bone-forming cells was found in areas of neoplastic bone formation (AIGNER *et al*. 2000).

Transchondroid osteogenesis is observed during endochondral osteogenesis in the epiphyseal growth plate; during bone fracture healing (SCAMMELL & ROACH 1996); in heterotopic osteogenesis induced by demineralized bone matrix or purified Bone Matrix Proteins (BMP) (KAWAKAMI *et al*. 1998, 1999; KAWAKAMI 2001); in osteogenesis induced by some epithelial cells (WŁODARSKI 1985); or by grafted chondrocytes (MOSKALEWSKI & MALEJCZYK 1989). It is also observed in distraction osteogenesis (YASU *et al*. 1997; LI *et al* 1999).

The concept of transformation of chondrocytes into osteoblasts was presented by HOLTROP in 1966, although the supposition was forwarded by some authors previously at the end of 19th century (cit. by ADAMS & SHAPIRO 2002). Holtrop's classical works on transplantation of rib cartilage into muscle, combined with autoradiographic studies enabled this author to conclude that hypertrophic chondrocytes of epiphyseal cartilage survived in the graft and were converted into osteoblasts and osteocytes (HOLTROP 1972). HOLTROP (1967) also demonstrated that, contrary to common opinion, hypertrophic chondrocytes are metabolically active . Later it was demonstrated that chondrocytes of nasal septa produce hyaline cartilage which under normal conditions never underwent endochondral osteogenesis. Chondrocytes of the established MC615 cell line, when exposed to a microenvironment modified by the BMP, start to synthesize some elements of bone matrix: – collagen type I, alkaline phosphatase (TSAO & CHUACH 1988). They also begin to express some osteoblastic markers – Gla protein (BGP) or osteocalcin, (VALCOURT *et al*. 1999).

A common progenitor cell for cartilage and bone

There is a body of evidence for the existence of a progenitor cell common for chondro- and osteogenesis (the so called osteochondral progenitor), and for its ability to switch from a chondrocyte phenotype into an osteoblast phenotype (HALL 1972; SIMMONS & KAHN 1979; YOO & JOHNSTONE 1998; HILL *et al*. 2005). ISHIZAKI *et al*. (1996), working on embryonic chondrocytes cultured *in vitro,* have shown the transition of chondrocytes into cells with osteoblastic markers. Chondrogenic progenitors of embryonic mandibular condyles cultured *in vitro* proliferate and differentiate into hypertrophic chondrocytes. The cartilage matrix stains metachromatically with toluidine blue (a characteristic feature of cartilage), whilst collagen type I and fibronectin – elements of bone matrix – become detectable (WEISS *et al*. 1986). The appearance of these markers suggests the presence of osteochondral progenitors in the embryonal mandibular condyles, which initially reveal their chondrogenic potency by deposition of cartilaginous matrix. This later mineralizes and the chondrocytes become hypertrophic. At a later stage bone matrix is formed either by osteochondral progenitor cells, or by transdifferentiated hypertrophic chondrocytes. The latter mechanism assumes another experimental model. ZERGA *et al*. (1999) have shown the role of parathormone or parathormone-related protein in the conversion of hypertrophic chondrocytes into osteoblasts, whilst KAVUMPURATH and HALL (1990) established that the conversion of chondrocytes into hypertrophic chondrocytes is thyroxine dependant.

The popular opinion is that the fate of (=hypertrophic) chondrocytes in endochondral osteogenesis is either degeneration and death, or apoptosis. When this happens, the osteoprogenitor cells invade emptied chondrocytic lacunae and start to

differentiate into bone-forming cells. However, the conversion of chondrocytes into osteoblasts is a possibility worth considering. This has been reported by KRELIN and KOCH (1965) who examined murine symphysis pubis cartilage cultured *in vitro*; by HOLTROP (1967) in mouse epiphyseal rib cultures, and by HALL (1972), in avian cartilage cultures. Based on the staining properties of matrix, its birefringence and on ultrastructure analysis of collagen fibers in perichondrium-free epiphyseal cartilage grafts onto allantoic membrane of chick embryos, KAHN and SIMMONS (1977) postulated the synthesis of bone matrix by chondrocytes, and the transformation of chondrocytes into bone cells. It was shown later that in the avian epiphyseal cartilage, some chondrocytes divide asymmetrically, one daughter cell enters apoptotic destruction, whilst the second remains alive and enters the cell cycle, proliferates and is gradually converted into osteoblasts (ROACH 1992, 1997; ERENPREISA & ROACH 1996; ROACH & ERENPREISA 1996).

However, the evidence for the transition of hypertrophic chondrocytes into osteoblasts is circumstantial. Proof for such a transition would be to mark permanently and selectively the hypertrophic chondrocytes, and follow their fate. Due to technical limitations, such an idea remains unrealistic at present.

Hypertrophic chondrocytes as metabolically active cells

In addition to the majority of dying hypertrophic chondrocytes, there is a proportion of cells able to synthetise DNA and re-enter a proliferation cycle, as shown by the incorporation of tritiated thymidine. Such hypertrophic chondrocytes express alkaline phosphatase, synthesize collagen type I and a number of non-collagenous bone proteins such as osteoclacin, osteopontin and osteonectin (ROACH 1992, 1997).

Hypertrophic chondrocyte activity is regulated by calcitropic hormones (PTH) and by locally secreted growth factors, e.g. Fibroblast Growth Factor (GERSTENFELD & SHAPIRO 1996). They express transforming growth factor-beta 1 (KAWAKAMI *et al*. 1999). Avian hypertrophic chondrocytes cultured in suspension transcribe osteopontin genes, simultaneously maintaining transcription of chondrocytic markers (CASTAGNOLA *et al*. 1991). In adherent cultures chondrocytes change their spherical morphology into spindle- or star-like shape and start to synthesize alkaline phosphatase, and secrete bone matrix protein which stain with Alcian blue. Once these cells reach confluency, mineralization of this matrix begins (DESCALZI CANCEDDA *et al*. 1992). GALOTTO *et al*. (1995) demonstrated the expression of osteoblast specific antigens in hypertrophic chondrocytes. LIAN *et al*. (1993) reported that the synthesis of bone-specific proteins, osteopontin and osteocalcin, by hypertrophic chondrocytes is related to mineralization of cartilaginous matrix. Hypertrophic chondrocytes produce a different type of chondroitin sulphate than nonhypertrophic ones, facilitating cartilage matrix mineralization (CARINO *et al*. 1985).

The secretion of matrix vesicles, the foci for mineralization, by hypertrophic chondrocytes was recently reported (ADAMS & SHAPIRO 2002). Cbfa1/Runx2, the earliest transcriptional regulator of osteoblast differentiation, is also expressed in hypertrophic chondrocytes and is involved in transdifferentiation of chondrocytes into bone cells (KARSENTY 2001).

On the basis of their own experiments and literature, DESCALZI CANCEDDA *et al*. (1992) proposed the conversion of hypertrophic chondrocytes into bone cells as an additional, third stage of chondrocyte differentiation. In the epiphyseal plate endochondral osteogenesis, the potential of hypertropic chondrocytes to become osteogenic cells is restricted exclusively to the border of cartilage and bone (GALOTO *et al*. 1995). Type III hypertrophic chondrocytes transdifferentiate into osteoblasts following a DNA synthesis phase. At the osteochondral junction of the epiphyseal plate, the osteogenic competence of hypertrophic chondrocytes is expressed almost simultaneously as in persisting osteoprogenitor cells.

According to the DESCALZI CANCEDDA *et al*. (1992) model, endochondral bone formation must be regarded as a joint endeavor of both chondrocyte-derived osteoblast-like cells (chondrocyte type III) and of osteoblasts. Therefore osteocytes entombed in the osteoid would be derived from both cell populations. They postulate that the process is triggered by interaction between stage III hypertrophic chondrocytes and osteoblastic progenitors via secretion of paracrine activators and mineralized extracellular matrix deposited by the opposing cell population (CANCEDDA *et al*. 1995). These hypertrophic chondrocytes having the potential to differentiate into osteogenic cells (type III chondrocytes), localized on the border of cartilage and osteogenic cells, are called the "borderline chondrocytes" (BIANCO *et al*. 1989).

It is worth mentioning that transdifferentiation of chondrocytes is not limited to hypertrophic cells. In *in vitro* conditions, chondrocytes, without signs of hypertrophy, can transdifferentiate into cells possessing osteogenic characteristics (HALL 1972; KAHN & SIMMONS 1977; ISHIZAKA *et al*. 1996; HEGERT *et al*. 2002; SONG & TUAN 2004). Present concept of models of intramembranacea

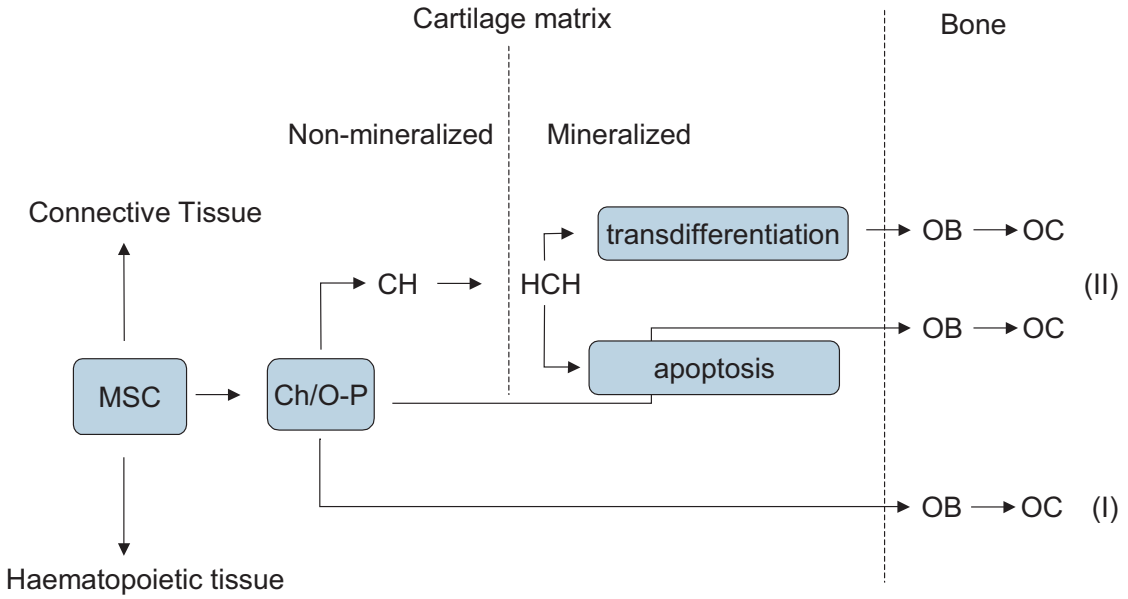

Fig. 1. Diverse mechanisms of osteogenesis. Endochondral osteogenesis involves chondrocyte metaplasia. Details in the text below.

osteogenesis (I) and of endochondral osteogenesis (II), which involves transdifferentiaton of hypertrophic chondrocytes into osteoblasts is shown on Figure 1.

Mesenchymal stem cells (MSC) differentiate towards all types of connective tissues, including skeletal. Cartilage and bone progenitor cells (Ch/O-P) enter the differentiation path leading to histogenesis of cartilage and /or bone.

In the model of osteogenesis intramembranacea (I) the Ch/O-P differentiate towards osteoblastic cells (OB) which, after completion of osteoid element secretion are embedded within mineralising matrix and become osteocytes (OC).

In the model of endochondral osteogenesis (II), the Ch/O-P cells differentiate towards chondroblast and chondrocytes (CH), the latter maturing into hypertrophic chondrocytes (HCH). Terminally differentiated HCH either degenerate or undergo apoptosis, but some transdifferentiate into cells able to synthesize elements of bone matrix – OB cells. Almost simultaneously the Ch/O-P cells, delivered with blood elements, colonize empty chondrocytic lacunae and in the medium provided by mineralised cartilaginous matrix, they differentiate toward OB and OC cells.

It is also possible that a transdifferentiation of non-hyperthrophic chondrocytes (CH) into OB cells can also occur.

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