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Distribution of CD105 and CD166 positive cells in the proximal epiphysis of developing rat humerus

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Summary. The expression of cell surface receptors, CD105 and CD166, are characteristic of mesenchymal stem cells in cartilage. However, there is limited data regarding their immunolocalization in the cartilage of developing rat epiphysis. The purpose of this study was to determine the presence of CD105 and CD 166 positive cells in the proximal epiphysis of developing rat humerus and specify their zonal distribution with age. The tissues of rat humerus were taken on embryonic day 15 (E15), embryonic day 19 (E19), postnatal day 10 (PN10), postnatal day 20 (PN20) and adult rats and studied for the immunolocalization of CD105 and CD166. Our results showed that CD105 and CD166 positive cells were scattered in early stages of development of humerus epiphysis. For E15, only the hypertrophic zone was positive, whereas for E19 almost all zones of the epiphysis were positively stained for these markers. For PN10 and PN20, the CD105 and CD166 positive cells were mainly localized on the surface of the articular cartilage. In adult articular cartilage the CD105 and CD166 positive cells were localized in the superficial and transitional zones and in the upper regions of the deep zone. Our study provides evidence that in the developing cartilage tissue the localization of CD105 and CD166 positive cells is both dynamic and stage dependent, which may imply the existence of stem cell-like cells in cartilage from an early age to adult.

Keywords: Epiphysis, Cartilage, Stem cells, CD105, CD166, Immunohistochemistry, Rat

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Introduction

The aggregation of chondroprogenitor mesenchymal cells, known as chondrogenic nodules, is the first sign of development of cartilage tissue. With the differentiation of chondrogenic nodules into chondroblasts, there is initiation of synthesis of the cartilage matrix. The chondroblasts progressively scatter with the increasing deposition of matrix. The chondroblasts further differentiate into mature chondrocytes, which are terminally differentiated cartilage tissue cells completely surrounded by matrix material (Ross and Pawlina, 2005). Chondrocytes, like all cells in connective tissue, derive from mesenchymal stem cells (MSCs) whose progeny also include tendon cells, bone marrow stromal cells, adipocytes, and osteoblasts (Caplan, 1991; Caplan et al., 1997). MSCs were originally identified in the non-hematopoietic compartment of bone marrow, which exhibits multi-lineage differentiation capacity and great expansion capacity *in vitro* (Friedenstein et al., 1976; Prockop, 1997; Pittenger et al., 1999). Human MSCs are characterized by the expression of specific cell surface antigens. Several antibodies have been generated for the characterization of these cells; Stro-1, SB-10 (specific for CD166), SH-2 (specific for endoglin/CD105), SH-3 and SH-4 (specific for CD73) (Simmons and Torok-Storb, 1991; Haynesworth et al., 1992; Bruder et al., 1997) CD146, CD90, CD44, and CD29, among others (Kolf et al., 2007).

(Alsalameh et al., 2004) identified a subpopulation of CD105 and CD166 positive cells in human articular cartilage, which are capable of differentiation into chondrocytes, suggesting that these markers could be specific for chondrogenic-lineage. This suggestion has been supported by others; Majumdar et al. (2000) observed that CD105 positive progenitor cells, when isolated from bone marrow, differentiate into chondrocytes (Majumdar et al., 2000). Arufe et al. (2009) reported that the cellular subset of differentiation of synovial CD105 positive MSCs has a chondrogenic capacity (Arufe et al., 2009). It has also been reported

that CD166 positive cells from the perichondrium have the ability to differentiate into chondrocytes (Arai et al., 2002). Studies regarding the molecular markers of articular cartilage stem cells have determined that CD105 and CD166 positive MSCs do not express chondrocyte specific markers, such as type II collagen and CDMP-1, but these cells are able to differentiate into chondrocytes, osteoblasts and adipocytes under appropriate conditions (Hiraoka et al., 2006; Ozbey et al., 2009).

During development, the human skeleton is mostly cartilaginous and is subsequently replaced mainly by bone. Adult articular cartilage lacks nerves, blood and lymph vessels, and has a high matrix to cell ratio (Dowthwaite et al., 2004). It contains only cells of mesenchymal lineage that are responsible for production and maintenance of the tissue (Dowthwaite et al., 2004; Hiraoka et al., 2006).

Mature articular cartilage is structurally arranged in four zones; the superficial, the transitional, the deep and the calcified. The cells of the superficial zone are flattened and elongated and have reserve stem cell potential with dividing and regenerative capacity (Dowthwaite et al., 2004; Hunziker et al., 2007). *In vivo* studies also show that the superficial zone is responsible for the appositional growth of articular cartilage (Archer et al., 1994; Hayes et al., 2001). Some cells in the superficial zone are derived by the division of progenitor cells which retain stem cell potential in this zone (Archer et al., 1994). The progenitor cells divide by mitosis to produce either progenitor stem cells in horizontal (lateral) and vertical (parallel to long axis) planes or support the terminal differentiation in the transitional and upper deep zones. Articular cartilage acts not only as an articulating layer but also as articular surface growth plate (Hunziker et al., 2007).

The growth plate is responsible for longitudinal growth of bones. The mammalian growth plate is composed of five principal zones; resting, proliferation, hypertrophic, calcification and ossification zones. Chondrocytes in the resting zone are irregularly scattered in a bed of cartilage matrix and then arranged into columns parallel to the axis of the bones in the proliferation and hypertrophic zones (Hunziker, 1994). (Abad et al., 2002) suggesting that the growth plate, especially in the resting zone, might contain stem cell-like cells capable of generating new clones of chondrocytes in the proliferation zone.

In this study we hypothesize that chondroprogenitor stem cell markers exist in prenatal ages to adulthood. Therefore, the aim of this study was to investigate the localization of stem cell markers, namely CD105 and CD166, in the developing rat epiphysis cartilage tissue and compare them to adult epiphysis cartilage.

Materials and methods

Animals

Thirty adult female and twelve adult male rats (*Rattus norvegicus*) were obtained from Akdeniz University Central Animal Service. Groups of two females and one male were caged together overnight. If a sperm-positive vaginal smear was observed on the following morning it was considered to indicate successful copulation. The sperm-positive day was designated as day 0 of pregnancy. Pregnant rats were killed by cervical dislocation after ether anesthesia and the day 15 (E15) and the day 19 (E19) embryos were removed by ceaserian section and epiphysis cartilage dissected with fine scissors. Epiphysis cartilage tissues were dissected on postnatal day 10 (PN10), postnatal day 20 (PN20) and from adult rats (6 months) under ether anesthesia followed by cervical dislocation with coarse and fine scissors as needed. The experimental protocol was approved by the animal care and usage committee of Akdeniz University (approval no: 01-11/01) and was in accordance with the declaration of Helsinki and The International Association for the study of pain guidelines.

Tissue processing

The tissues from E15, E19 and PN10 were fixed by immersion in 10% buffered formalin and then processed without decalcification for embedding in paraffin wax for histochemical and immunohistochemical investigations. Tissues from PN20 and adult rats were decalcified before embedding in paraffin; epiphysis cartilage tissues were fixed by immersion in 10% buffered formalin for 1 week. Then tissues were washed in tap water for 24 h, decalcified in 25% formic acid (Merck, 1002649026, Darmstadt Germany, pH 2.2) for 3 days (Skinner et al., 1997; Miyanishi et al., 2005; Ozbey et al., 2009). That process was followed by several washes under tap water for 24 h and then the tissues neutralized in 0.35 M sodium sulphate (304720401, Lachema Co., Brno, Czech Republic) solution for 3 days and finally embedded in paraffin wax. All samples were serially sectioned at 5-μm thickness by Microtome (Leica, RM2125RT, Germany) and mounted onto poly-L-lysine coated slides (Sigma-Aldrich, St. Louis, MO, USA). For drying applications, the slides were placed into an Oven Incubator (38°C) overnight before routine light histological examination (Hematoxylin-Eosin,H-E) and immunohistochemical labeling.

Immunohistochemistry

For immunohistochemical labeling, 5 μm tissue sections were deparaffinized in xylene and sections were rehydrated through a decreasing gradient of ethanol (100%, 90%, 80% and 70% for 5 minutes, respectively). The antigen retrieval was achieved by overnight incubation of the slides in boric acid at 57°C (Wilson et al., 2007). Boric acid was prepared with phosphate buffered saline (PBS) containing 2.1% boric acid (Merck 0149654, Darmstadt, Germany). After rinsing 3 times in PBS, endogenous peroxidase activity was blocked by 3% hydrogen peroxide in methanol for 15 min and three

rinses in PBS. For the reduction of nonspecific background staining, the sections were incubated with Ultra V Block for 10 minutes at room temperature. The need to match species with the secondary antibody is eliminated due to the lack of normal serum in Ultra V Block (Lab Vision Corporation, TA-125UB, Fremont, CA, USA). Rabbit polyclonal anti-human CD105 (H-300, SC-20632) and rabbit polyclonal anti-human CD166 (H-108, SC-25624) primary antibodies (Santa Cruz Biotechnology Inc, Santa Cruz, CA) were applied as 1:100 and 1:200 dilutions overnight at +4°C, respectively. After several washes in PBS, antibodies were detected using an avidin-biotin horseradish peroxidase complex (Ultra Vision Detection System Anti-Polyvalent, Alk-Phos, Fremont, CA, USA) for 30 minutes at room temperature. The sections were rinsed with PBS, and antibody complexes were visualized after incubation with 3,3'-diaminobenzidine tetrahydrochloride (DAB, Bio-Genex, San Ramon, CA.) resulting in a brown precipitate. Sections were counterstained with Harris Haematoxylin (Sigma-Aldrich, St. Louis, MO) to enable visualization of nuclei then dehydrated (rinsing the slides with 70%, 80%, 90% and 100% ethanol for 5 minutes, respectively) and mounted under glass coverslips in DPX (BDH Laboratories, Poole, UK). Immunohistochemistry was performed on three independent samples with identical results. Negative control sections for each antibody were performed by replacing the primary antibody with normal rabbit IgG (Santa Cruz Biotechnology, SC: 2051) for all samples at the same concentrations as the primary antibodies. In all cases no signal was observed. Photomicrographs were taken with an Axioplan microscope (Zeiss, Oberkochen, Germany).

Semi-quantitative evaluations using H-SCORE

The evaluations of the immunohistochemical labeling in all groups were performed utilizing H-SCORE (Sahin et al., 2005). Labeled sections were evaluated using an Axioplan microscope (Zeiss, Oberkochen, Germany) with a special ocular scale. From three randomly selected slides, five different fields at x 200 magnification were analyzed for immunohistochemical staining of the antibodies. The evaluations were recorded as percentages of positive stained cells of all types in each of four intensity categories which were denoted as 0 (no labeling), 1+ (weak labeling), 2+ (distinct labeling), 3+ (intense labeling). For each cell type, an H-SCORE value was derived by counting the percentages of cells that stained at each intensity, multiplied by the weighted intensity of the staining [H-SCORE = Sigma(∑) P_i (i+1), where i is the intensity score and P_i is the corresponding percentage of the cells]. The H-SCORE values were presented graphically. The H-SCORE was performed by two independent observers and interobserver variation was 5%.

Statistical Analysis

The data from the H-SCORE were analyzed with non-parametric Kruskal-Wallis ANOVA by rank, followed by a *post hoc* Student-Newman-Keuls test. Values are presented as mean ± Standard Deviation (SD). Statistical calculations were performed using Sigma Stat for Windows, version 3.0 (Jandel Scientific Corp. San Rafael, CA). Statistical significance was defined as $p<0.05$.

Results

The development of the proximal epiphysis of the rat humerus

In the E15 group, anterior extremity anlagen were observed. At this stage of development, cartilage primordium was located in peripheral embryonic tissue and did not exhibit ossification. Some hypertrophic cells were also visible (Fig. 1a). In the E19 group, joint cavity and the zones of the epiphysis were observed in the humerus. As the primary ossification center developed the formation of cartilaginous growth plate occurred

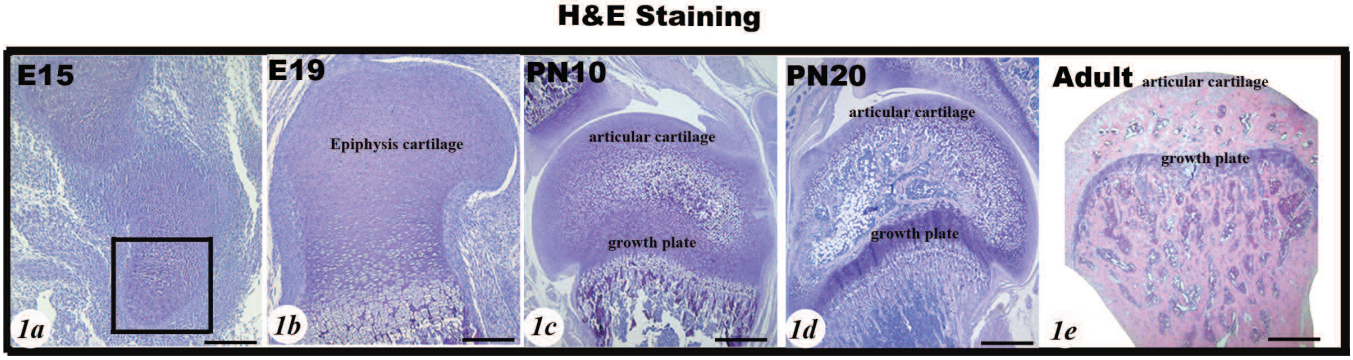

Fig. 1. Developing epiphysis cartilage tissue architecture in Hematoxylin-Eosin stained sections. **a.** E15, square area defines the future humerus head, **b.** E19. **c.** PN10. **d.** PN20. **e.** Adult. Scale bar: 400 μm.

(Fig. 1b). In the PN10 group, joint cavity, secondary ossification center and all zones of the growth plate were developed (Fig. 1c). In the PN20 group, the secondary ossification center was the major part of the epiphysis and the zones of the growth plate were obvious (Fig. 1d). During the adult stage, the growth plate presented as a very thin layer appearing between the secondary ossification center and the metaphysis region (Fig. 1e).

Immunohistochemical results

The immunostaining intensity of CD105 and CD166 in the developing and adult rat humerus were evaluated semi-quantitatively and summarized in Tables 1 and 2, respectively. Briefly, in articular cartilage, CD105 and CD166 staining were in the surface and transitional zones on E19. With the progression of development, on PN10 and PN20, the staining was mainly in the transitional and deep zones. In adult, the CD105 and CD166 staining were apparent from the surface zone to the deep zone. In the growth plate, the major staining for CD105 and CD166 were on E19, in the resting and hypertophic zones (Tables 1, 2).

CD105

In the E15 group, CD105 positive cells were mainly located in peripheral mesenchymal connective tissue (stars) and perichondrium (double arrow). A few positive cells were also detected in hypertrophic cells of

Table 1. Semi-quantitative distribution of CD105 immunoreactivity in proximal epiphysis of developing rat humerus.

Epiphysis cartilage		E15	E19	PN10	PN20	Adult
Articular Cartilage Zones	Surface zone	θ	++	(+)	(+)	+++
	Transitional zone	θ	++	++	+++	+++
	Deep Zone	θ	θ	+++	+++	++
	Calcification Zone	θ	θ	(+)	0	0
Secondary Ossification Center		θ	θ	++	++	+++
Growth Plate Zones	Resting Zone	θ	++	+	(+)	0
	Proliferation Zone	θ	(+)	(+)	(+)	0
	Hypertrophic Zone	++	++	+	+	0
	Calcification Zone	θ	(+)	0	0	0
	Ossification Zone	θ	+++	+++	+++	+++

E: Embryonic day; PN:Postnatal day; 0: Negative; (+): Weak; +: Moderate; ++: Strong ; and +++: Very strong, θ: the area has not developed

Table 2. Semi-quantitative distribution of CD166 immunoreactivity in proximal epiphysis of developing rat humerus.

Epiphysis cartilage		E15	E19	PN10	PN20	Adult
Articular Cartilage Zones	Surface zone	θ	+++	+	(+)	+++
	Transitional zone	θ	+++	+++	++	+++
	Deep Zone	θ	θ	++	++	++
	Calcification Zone	θ	θ	(+)	0	0
Secondary Ossification Center		θ	θ	++	++	++
Growth Plate Zones	Resting Zone	θ	++	+	0	0
	Proliferation Zone	θ	(+)	+	0	0
	Hypertrophic Zone	+	+++	++	(+)	0
	Calcification Zone	θ	(+)	0	0	0
	Ossification Zone	θ	+++	+++	+++	+++

E: Embryonic day; PN:Postnatal day; 0: Negative; (+): Weak; +: Moderate; ++: Strong ; and +++: Very strong, θ: the area has not developed

the growth plate (arrows) (Fig. 2a).

In the E19 group, CD105 immunostaining was detected in the surface and transitional zones of articular cartilage (Fig. 2b), and also in the resting, calcification and ossification zones of the growth plate (Fig. 2c, Table 1).

In the PN10 group, CD105 positive cells were mainly in the transitional and deep zones of articular cartilage. In the growth plate, the resting and hypertrophic zone cells were positive for CD105 (Fig. 2d-g, Table 1).

In the PN20 group, the positive cells were mostly located adjacent to the articular cavity. During this stage, very strong CD105 immunostaining was observed in the transitional and deep zone cells of articular cartilage (Fig. 2h, Table 1). The hypertrophic zone cells were also positive for CD105 (Fig. 2i, Table 1).

In the articular cartilage of adult rat humerus, CD105 positive cells were in the surface, transitional and the upper part of the deep zones. The calcification zone cells were negative for CD105 (Fig. 2j, Table 1). There was no CD105 immunostaining in the growth plate (Fig. 2k).

When comparisons were made between groups, H-SCORE analysis revealed that the intensity of labeling for CD105 on E19 and PN10 was significantly higher than on E15. In the adult articular cartilage, there was a significant decrease in CD105 immunostaining compared to E19 and PN10 (Fig. 4).

CD166

In the E15 group, CD166 immunolabeling showed a similar pattern to that observed for CD105; the positive cells were mainly located in peripheral mesenchymal connective tissue (stars), perichondrium (double arrow) and hypertrophic cells (arrows) (Fig. 3a).

In the E19 group, CD166 positive cells were located

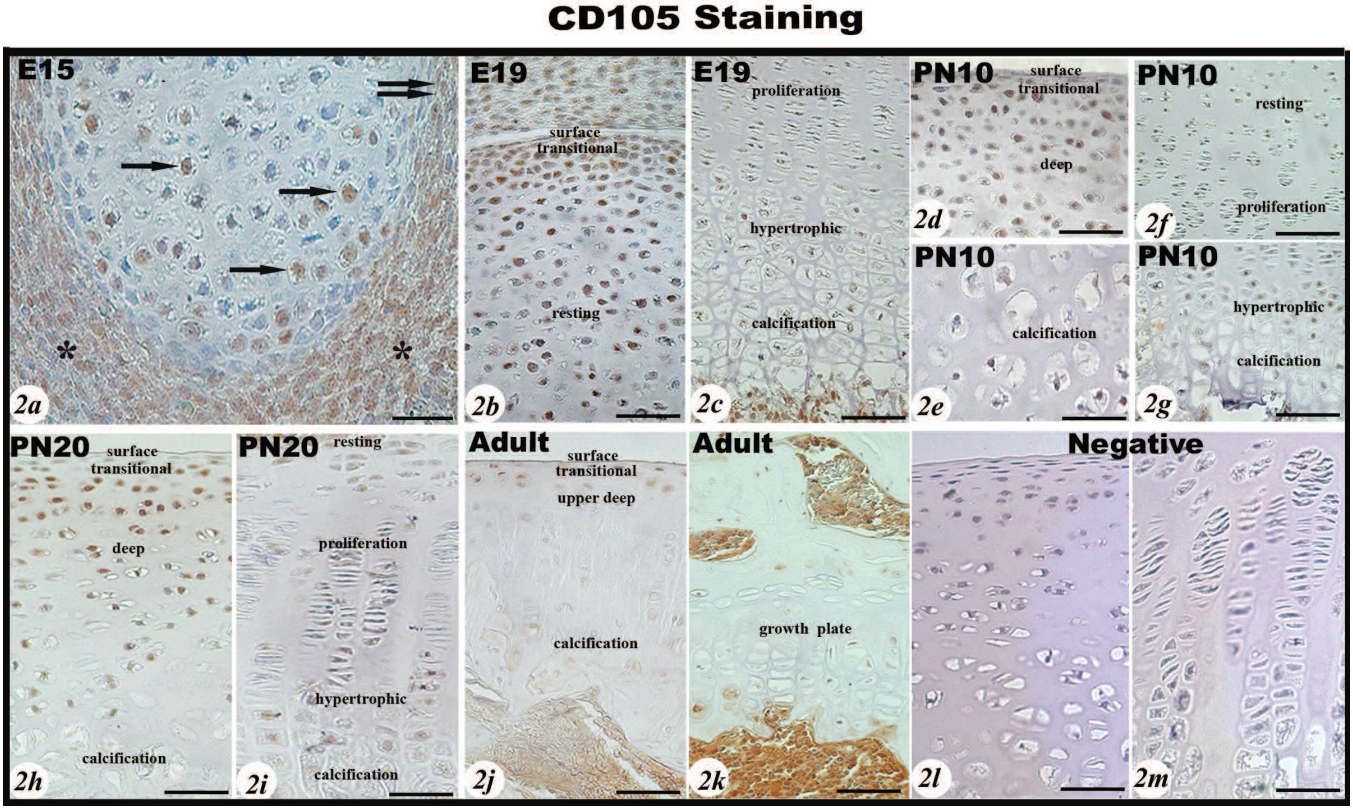

Fig. 2. CD105 immunostaining in developing and adult epiphysis cartilage **a.** E15; CD105 immunostaining in peripheral mesenchymal connective tissue (asterisks), perichondrium (double arrows) and hypertrophic cells (arrows). **b.** E19; the surface, transitional and resting zone stainings are shown. **c.** E19; CD105 immunostaining in the proliferation, hypertrophic and calcification zones. **d, e.** PN10; CD105 immunolabeling in the surface, transitional, deep and calcification zones of articular cartilage. **f, g.** PN10; CD105 immunolabeling in the resting, proliferation and hypertrophic zones of the growth plate. No immunostaining in the calcification zone. **h.** PN20; CD105 staining in the surface, transitional, deep zones. No immunostaining in the calcification zone. **i.** PN20; the growth plate stainings in the resting, proliferation, hypertrophic zones. No immunolabeling in the calcification zone. **j.** Adult; CD105 immunopositive cells in the surface, transitional and upper deep zones. **k.** Adult; no immunopositivity in the growth plate. **l, m.** PN20; No immunolabeling in negative control sections. Scale bars: a, 25 μm; b-m, 50 μm.

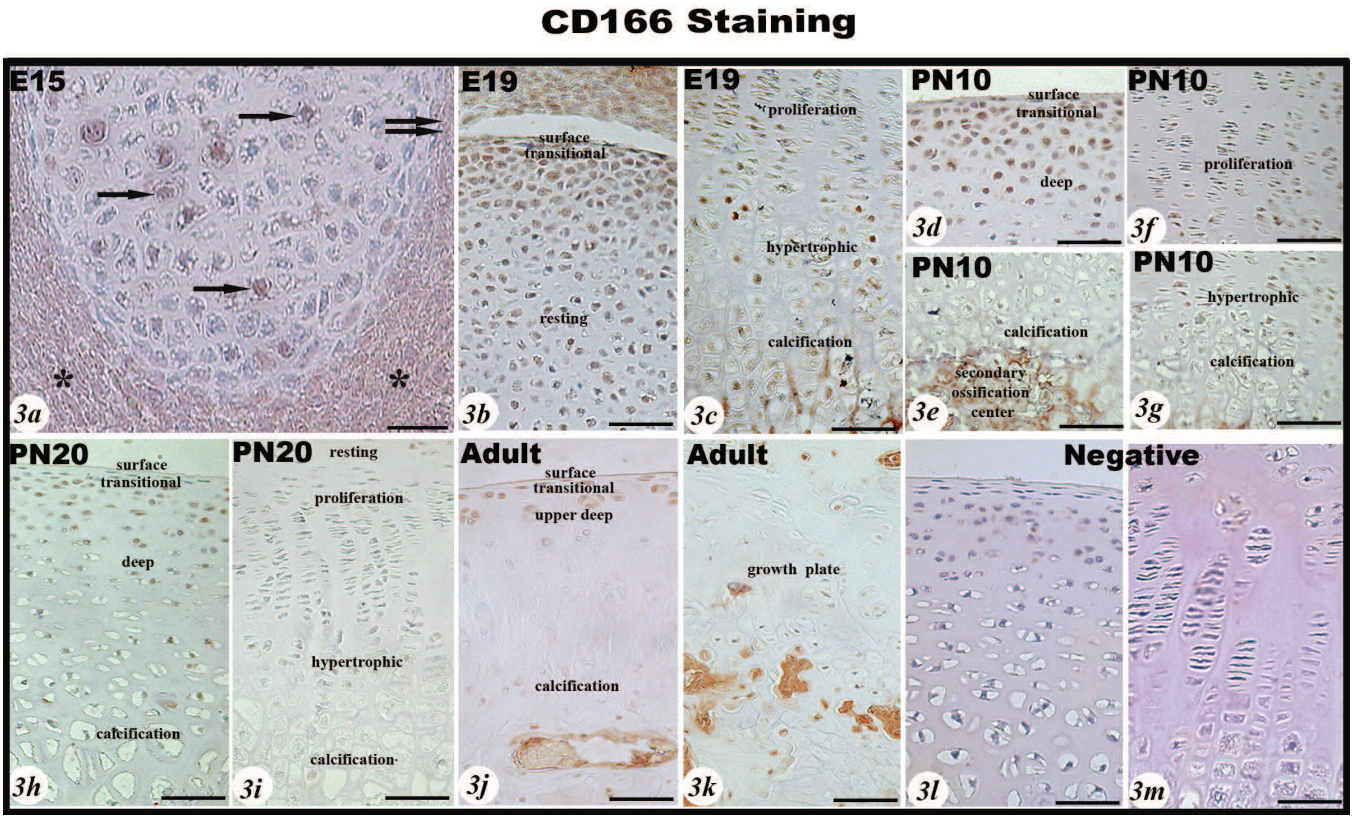

Fig. 3. CD166 immunostaining in developing and adult epiphysis cartilage **a.** E15; CD166 stainings in peripheral mesenchymal connective tissue (asterisks), perichondrium (double arrows) and hypertrophic cells (arrow). **b.** E19; Strong stainings are seen close to the joint cavity and in the surface, transitional and resting zones. **c.** E19; CD166 immunostaining in the proliferation, hypertrophic and calcification zones. **d, e.** PN10; CD166 immunolabeling in articular cartilage of the surface, transitional, deep, calcification zones and in the secondary ossification center. **f, g.** PN10; CD166 immunolabeling in the proliferation and hypertrophic zones of the growth plate. The calcification zone is negative. **h.** PN20; strong immunolabeling is seen close to the joint cavity in the articular cartilage. CD166 staining in the surface, transitional and deep zones. The calcification zone is negative. **i.** PN20; In the growth plate, weak immunostaining in the hypertrophic zone. No reactivity in the resting, proliferation and calcification zones. **j.** Adult; the surface, transitional and upper deep zone stainings are shown. The calcification zone is negative for CD166. **k.** Adult; no immunopositivity in the growth plate. **l, m.** No immunolabeling in the negative control sections. Scale bars: a, 25 μm; b-m, 50 μm.

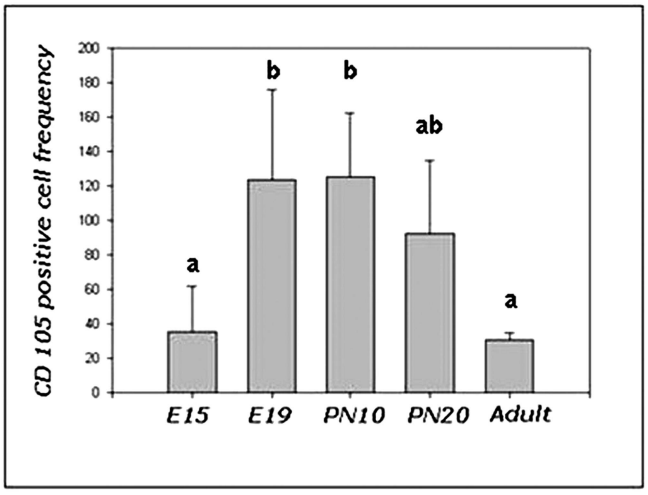

Fig. 4. CD105 positive cell frequency in epiphysis of developing rat humerus. On E19 and PN10, the staining cell frequency was significantly higher than on E15 and adult. Groups marked with the same letter do not differ significantly.

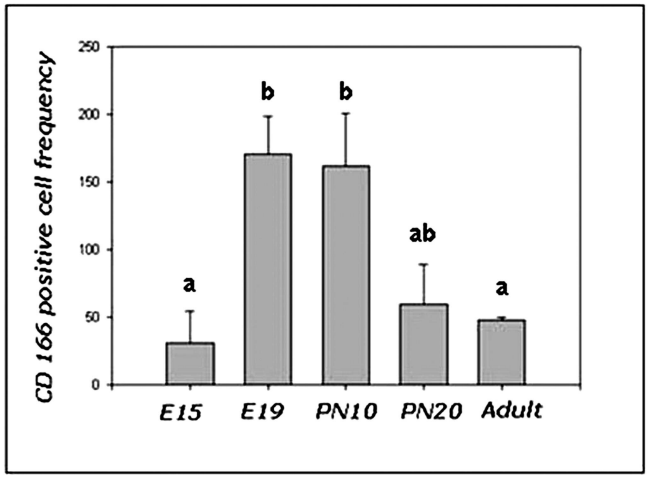

Fig. 5. CD166 positive cell frequency in epiphysis of developing rat humerus. On E19 and PN10, the staining cell frequency was significantly higher than on E15 and adult. Groups marked with the same letter do not differ significantly.

in the surface and transitional zones of the articular cartilage (Fig. 3b, Table 2). CD166 positive cells were mostly in the resting and hypertrophic zones of the growth plate (Fig. 3c, Table 2).

In the PN10 group, CD166 positive cells were mostly located adjacent to the articular cavity. The transitional and deep zone cells were mainly positive for CD166 in the articular cartilage There were also some positive cells in the surface zone (Fig 3d, Table 2). The secondary ossification center was positive for CD166 staining (Fig 3e). The proliferation zone also showed a moderate staining pattern (Fig 3f).In the growth plate, CD166 positive cells were mainly in the hypertrophic zone (Fig 3g).

In the PN20 group of articular cartilage, the immunostaining was positive in the transitional and deep zones. The calcification zone cells were negative for CD166 (Fig. 3h, Table 2). In the growth plate, a weak staining was observed in the hypertrophic zone (Fig. 3i).

In the articular cartilage of adult rat humerus, CD166 immunostaining was in the upper part of the articular cartilage. CD166 positive cells were in the surface, transitional and upper deep zones of articular cartilage (Fig. 3j, Table 2). There was no CD166 immunostaining in growth plate (Fig. 3k).

The H-SCORE analysis revealed that CD166 positive cell frequency in all groups was similar to CD105. The intensity of labeling for CD166 on E19 and PN10 was significantly higher than on E15 and adult (Fig. 5).

Controls

No immunolabeling was observed in the negative control sections (Figs. 2l,m, 3l,m).

Discussion

This study demonstrates that the localization of CD105 and CD166 positive cells in the developing rat cartilage tissue are dynamic and stage dependent. It also suggests the possibility that mesenchyme-like stem cells exist in cartilage from an early age to adult.

MSCs have a large capacity for self-renewal while maintaining their multipotency and can be characterized by the expression of specific markers. The monoclonal antibody SH-2 raised against human marrow-derived MSCs recognizes CD105 and the transforming growth factor ß (TGFß) receptor type III. CD105 on MSCs potentially plays a role in TGF-ß signalling in the control of chondrogenic differentiation of MSCs (Barry et al., 1999). Antibody SB-10 reacts with CD166 (Bruder et al., 1998). Although *in vivo* there is no unique marker for MSCs, the coexpression of CD105 and CD166 indicates the presence of MSCs (Majumdar et al., 1998). An inverse correlation between CD105 expression and the differentiation status of MSCs has also been reported (Yang et al., 2006; Delorme et al., 2008; Jin et al., 2009). These findings support the idea that the cells expressing these molecules are thought to have the property of stem cells.

In our study, CD105 and CD166 positive cells were observed on E15 in the area adjacent to the articular primordium, and in the hypertrophic cells; located in the middle of the humerus cartilage primordium. These positive cells may indicate the existence of MSCs during chondrogenesis. We observed CD105 and CD166 positive cells predominantly in the resting zone of the epiphysis cartilage on E19 (Abad et al., 2002) reported that the resting zone of the growth plate plays an important role in endochondral ossification due to the presence of stem cell-like cells. It is suggested that these cells in the resting zone provide clones of proliferative chondrocytes, due to the secretion of a growth plate-orienting factor that diffuses into the proliferative zone, setting up a concentration gradient that guides the orientation of proliferative columns which align parallel to a long bone model. They also play a role in the synthesis or regulation of the expressions of proteins including Indian Hedgehog, Bone Morphogenetic Proteins, Fibroblast Growth Factors, and retinoids which maintain the proliferation process in the proliferation zone and prevent them from differentiating until their number becomes sufficient. Our observations suggest the existence of CD105 and CD166 positive cells in all zones, particularly in the resting zone of the epiphysis cartilage on E19.

In adult articular cartilage, the immunolocalization of CD105 and CD166 occurs in the superficial and transitional zones as well as the upper deep zone, which faces the surface of the articular cartilage. These findings are similar to what has been described previously for rabbit and marsupial (Oreja et al., 1995; Hayes et al., 2001; Hunziker et al., 2007) indicating positive cells were located in the upper part of the articular cartilage. Other studies that focused on determining the chondrocyte proliferation rate in newborn rabbit articular knee cartilage with thymidine incorporation are consistent with our results, in that they suggest the presence of proliferative cells on the surface of the articular cartilage and just above the subchondral plate (Mankin, 1962, 1964; Lennon et al., 2000). Similar findings were reported by (Oreja et al., 1995) where they demonstrated that the superficial zone has proliferative activity in 6-week-old rabbits.

Our H-SCORE results show that positive cell frequencies for CD105 and CD166 were significantly higher on E19 and PN10 compared to E15 and adult. This pattern coincides with the designate aspects of chondrogenesis; on E15, the articular cartilage and growth plate zones have virtually no development, whereas on E19 and PN10 more development has occurred and mesenchymal stem cell-like cells have localized mainly in the upper region of the growth plate. Considering the completion of developmental process of chondrogenesis, it would be consistent to observe a decrease in the number of mesenchymal stem cell-like cells in adults.

Our results suggest that the cells which are positive for CD105 and CD166 in the articular cartilage may have an important role in vitality, cell-matrix interaction, migration and growth of epiphysis cartilage. Although it is accepted that adult cartilage tissue contains populations of cells that have the capacity for renewal, the emphasis of this study was to specifically investigate the possibility that CD105 and CD166 expressing cells could be the progenitor stem cell markers in cartilage. Thus, we suggest that CD105 and CD166 positive cells could be selected as a biomarker of cartilage stem/progenitor-like cells in *in vivo* studies.

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