DOI 10.26724/2079-8334-2021-2-76-198-202
UDC 616.71-018.3-018.2-007.17-092.9

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PECULIARITIES OF SYNOVIOCYTES AND CHONDROCYTES PROLIFERATIVE ACTIVITY IN RATS WITH EXPERIMENTAL MODEL OF UNDIFFERENTIATED DYSPLASIA OF CONNECTIVE TISSUE

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The purpose of the work was to study the dynamics of proliferative activity of the synovial membrane cells of the joint capsule and articular cartilage chondrocytes of rats in the postnatal period on the background of experimental model of undifferentiated dysplasia of connective tissue. It was formed in Wistar rats by means of subcutaneous injection with human immunoglobulin on the 18th day of fetal period of development according to the method elaborated by N. A.Voloshyn (1981). It was revealed, that during the first two weeks after birth in experimental rats proliferative activity of synovial membrane cells is significantly decreased in comparison with control. The distribution of ki-67+ cells in different zones of the articular cartilage of the distal epiphysis of rats' femur during the first month of life is different depending on the degree of morphological and functional differentiation of chondrocytes. In rats with experimental undifferentiated dysplasia of connective tissue the increased proliferative activity of articular cartilage chondrocytes in all morphofunctional zones reduces at the 11th, 14th days after birth, this is associated with earlier formation of subchondral bone (at the 11th day compared to the 14th in control group) and the change in the rate of differentiation and functional activity of chondrocytes.

Key words: knee joint, chondrocyte, synoviocyte, joint capsule, articular cartilage, undifferentiated dysplasia of connective tissue, Ki-67.

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ОСОБЛИВОСТІ ПРОЛІФЕРАТИВНОЇ АКТИВНОСТІ СИНОВІОЦИТІВ ТА ХОНДРОЦИТІВ ЩУРІВ З ЕКСПЕРИМЕНТАЛЬНОЮ МОДЕЛЛЮ НЕДИФЕРЕНЦІЙОВАНОЇ ДИСПЛАЗІЇ СПОЛУЧНОЇ ТКАНИНИ

Мета роботи полягала у вивченні динаміки проліферативної активності синовіоцитів суглобової капсули і хондроцитів суглобового хряща щурів в постнатальному періоді на тлі експериментальної моделі недиференційованої дисплазії сполучної тканини. Вона була сформована у щурів лінії Вістар шляхом підшкірного введення людського імуноглобуліну на 18-ту добу внутрішньоутробного періоду за методом М.А. Волошина (1981). Було встановлено, що протягом перших двох тижнів після народження у експериментальних щурів проліферативна активність синовіоцітів була достовірно знижена в порівнянні з контролем. Розподіл ki-67+ клітин в різних зонах суглобового хряща дистального епіфіза стегнової кістки щурів протягом першого місяця життя різниться в залежності від ступеня морфологічного та функціонального диференціювання хондроцитів. У щурів з експериментальною недиференційованою дисплазією сполучної тканини підвищена проліферативна активність хондроцитів суглобового хряща у всіх морфофункціональних зонах знижується на 11-ту, 14-ту добу після народження, що асоціюється з більш раннім формуванням субхондральної кістки (на 11-ту добу, в порівнянні з 14-ю добою в контрольній групі) і зміною в темпах диференціювання та функціональної активності хондроцитів.

Ключові слова: колінний суглоб, хондроцит, синовіоцит, капсула суглоба, суглобовий хрящ, недиференційована дисплазія сполучної тканини, Кі-67.

The study is a fragment of the research project "Morphological features of bone remodeling under conditions of their polysegmental damage and surgical correction," state registration No. 0120U103164.

Joint disorders represent one of the most common symptoms of undifferentiated dysplasia of connective tissue, which has a multifactorial nature. Different factors influencing fetal development cause abnormalities of connective tissue formation, maturation, change relation between its components and capacities of its fibers, extracellular matrix and cells' properties including proliferation, differentiation and synthetic activity. The nuclear antigen Ki-67 is strongly associated with cell proliferation and is expressed in G1, S, G2, M phases of the cell cycle but not in G0 phase and can be used to identify dividing cells. Antigen Ki-67 is a short-lived protein, collapsing over 1–1.5 hours, so Ki-67 is detected only in cells that divide, because it does not have time to accumulate and remain in the cells in the interphase stage [7]. Marker Ki-67, as a whole, reflects the proliferative activity of the tissue, without singling out certain populations of cells.

Ki-67 is involved in main structural transformations during the process of mitosis [8]. Ultrastructural studies have shown that it is an important component in the spindle formation, is essential

for the formation of perichromosomal layer in human and prevents adhesion of mitotic chromosomes [3, 9]. The expression of Ki-67 reflects the proliferative activity of stromal cells and resident and infiltrating dividing cells. Cell proliferation is closely connected with growth and is one of the main links of the mechanism. The intensity of proliferation of chondrocytes can vary depending on the number of dividing cells (proliferative pool) and the duration of their mitotic cycle [6]. Scientists from different countries [1, 2, 6] investigate temporal characteristics of the mitotic cycle of chondrocytes during cartilage formation in early ontogenesis in the epiphyseal cartilage of bones after birth. These studies helped to better define the mechanisms of longitudinal bone growth and the factors influencing this process.

It is necessary to clarify and add data on the kinetics of the synovial membrane cells and the articular cartilage chondrocytes proliferation in the early postnatal period.

The purpose of the work was to study the dynamics of proliferative activity of the synovial membrane cells of the joint capsule and articular cartilage chondrocytes of rats in the postnatal period on the background of experimental model of undifferentiated dysplasia of connective tissue.

Materials and methods. The work investigated the knee joints of 2 groups of rats from birth up to the 30th day of postnatal life. The first group consisted of rats Wistar, which on the 18th day of fetal period of development were subcutaneously injected with human immunoglobulin according to the method elaborated by N. A. Voloshyn (1981). The second group served as control rats, which on the 18th day of fetal period of development were subcutaneously injected with a saline solution. While working with experimental animals it was guided by the "European Convention for the protection of vertebrate animals used for experimental and other scientific purposes" (Strasbourg, 18.03.86). Animals were contained in standard conditions of vivarium according to Law of Ukraine № 1759–VI (15.12.2009) On the Protection of Animals from Cruelty. The experimental investigation of the knee joints was performed at the 1st, 11th, 14th, 30th day, following the order "On measures for further improvement of organizational forms of work using experimental animals".

For light microscopy histological samples were stained with hematoxylin and eosin. The immunohistochemical method used the monoclonal antibody ki-67 (LabVision, USA) to identify cells in phases M, S, G1 and G2 of the cell cycle. Sections of 5 μm thick were glued on the glass with a special adhesive coating, slices were deparaffinized. Unmasking of antigens was performed by heating sections in citrate buffer pH=6.0 on a water bath for 30 minutes at the temperature of 98–99^{0}C. Streptavidin-Biotin system of visualization of antibody LSAB2 (peroxidase label+benzidine) (LabVision, USA) was used. The relative number of ki-67+ cells in the synovial membrane of the joint capsule of the knee joint and in the morphofunctional zones of articular cartilage: in the peri-, pro- and metachondral zones of the fetal articular cartilage; in the superficial and intermediate zones of juvenile cartilage were counted. The index of proliferation was examined under immersion magnification after counting 1000 cells with the subsequent calculation of the percentage.

Data processing**.** Analysis of the obtained results was performed by means of statistical methods with the use of computer license program "Statistica for Windows 13" (StatSoft Inc., No. JPZ804I382130ARCN10-J). The compared results were considered significantly different at $p<0.05$, that is generally accepted for biological and medical researches.

Results of the study and their discussion. In newborn animals of all groups the layers of the joint capsule are not clearly differentiated, villi are not formed. Intra-articular surface of the synovial membrane of a newborn is uneven, forms shallow mounds and a few thin villi. Cells of the covering layer of the synovial membrane are of predominantly rounded shape with a moderate nuclear-cytoplasmic ratio. Ki-67$^+$ cells are detected in all parts of joint capsule (fig.1). The highest level of proliferative activity is determined in control group (14.00±0.80 %). Minimal proliferative activity is detected in the synovial membrane of rats with experimental undifferentiated dysplasia of connective tissue (0.86±0.40 %) (table 1).

In newborn rats of all groups the articular cartilage of the distal epiphysis of the femur is characterized as an embryonic one, the maximum relative number of ki-67+ cells is determined in metachondral zone (fig. 1).

In the control animals the index is highest compared to the experimental group (18.42±0.38 %). The highest density of ki-67+ cells in metachondral zone corresponds to a proportional growth of immature articular cartilage. Labeled cells are evenly distributed and are not united in isogenic groups.

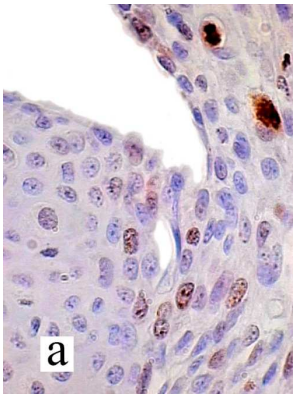
In prochondral zone, the maximum index of proliferation is determined in the control group of rats (12.86±0.80 %). In experimental newborn rats, the highest relative content of ki-67+ cells is determined in the perichondral zone of immature articular cartilage (9.43±0.80 %).

On the 11th day after birth in the synovial membrane of the of the rats' knee joint capsule villi begin to form. In control animals' synoviocytes of the synovial membrane-covering layer are uneven: in invaginated parts of synovial membrane, in the base of meniscus the synoviocytes are rounded, arranged in 1–2 rows, between these areas the synoviocytes are elongated along the longitudinal axis of the joint, being arranged also in 1or 2 ranges. There are some areas of the synovial membrane coating layer, filled with intercellular substance exhibiting moderate reaction in samples stained with alcyan blue with a critical concentration of magnesium chloride 0.6 M. In fibrous membrane of joint capsule cells, occupy 30.0±2.37 % of relative area. In control rats, in general, the proliferative activity of synovial membrane cells decreases in comparison with newborns (3.59±0.37 %), in the transition zone of joint capsule this index increases (of 8.21±0.74 %).

The proliferative activity of synovial membrane cells in rats with experimental undifferentiated dysplasia of connective tissue in general does not change compared to newborns (0.57±0.40 %), while in the transition zone it is significantly reduced (0.86±0.40 %) and becomes significantly lower than in controls.

On the 11th day after the birth in rats with experimental undifferentiated dysplasia of connective tissue the articular cartilage, in contrast to control rats, is determined as juvenile, with defined superficial, intermediate and basal zones, subchondral bone is formed. The proliferative activity of cells is reduced compared to newborn rats; this may indicate the prevalence of differentiation processes of chondrocytes on the proliferation. This fact is confirmed by a significant increase in the size of chondrocytes of the intermediate zone of rats with experimental undifferentiated dysplasia of connective tissue (126.01±5.23 μm^2) compared to chondrocytes prochondral zone of articular cartilage in control animals (78.72±3.29 μm^2).

On the 14th day after birth, articular cartilage in all groups of rats corresponds to the juvenile one. In the articular cartilage, one can define the superficial, intermediate and basal zones. The basal zone is represented by chondrocytes with vacuolated cytoplasm. In rats with experimental undifferentiated dysplasia of connective tissue, the proliferative activity of chondrocytes of the superficial zone is minimal when compared to the control (1.00±0.37 % and 8.16±1.14 %, respectively) (fig. 2). In the intermediate zone of articular cartilage the same trend is maintained (1.25±0.37 percent and 6.25±0.74 %, respectively, in experimental and control animals). In the basal zone of the articular cartilage of all groups of rats, ki-67$^+$ cells are not detected. Low proliferative activity of chondrocytes of articular cartilage in rats with experimental undifferentiated dysplasia of connective tissue is determined on the background of a significant increasing of articular cartilage thickness (619.41±11.41 μm) compared with the control animals (397.14±9.95 μm). In rats with experimental undifferentiated dysplasia of connective tissue, the increase of intermediate zone chondrocytes size (175.89±10.20 μm2) compared to the control animals (104.49±3.78 μm^2) is also determined. The increase of this index is associated with more pronounced cytoplasmic vacuolization of chondrocytes compared to the control.



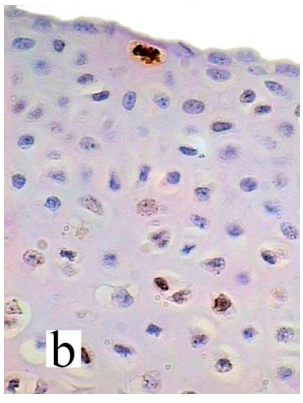
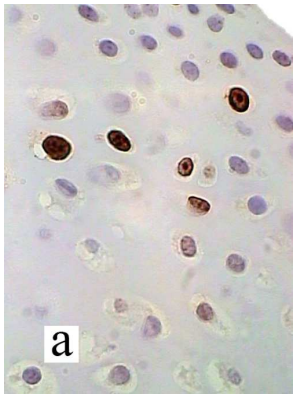


Fig. 1. The 1st day after birth, control rat, distribution of Ki-67+ cells. a) Intermediate zone of synovial membrane b) Articular cartilage. x1000.



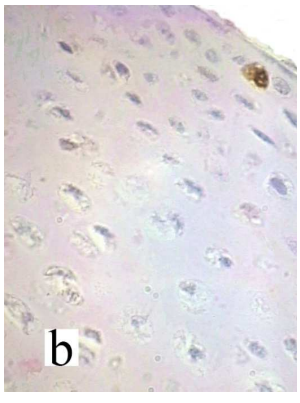


Fig. 2. Distribution of Ki-67+ cells in articular cartilage of a) control rat b) experimental rat at the14th day after birth. x1000.

At the 30th day after birth, the covering layer of the joint capsule synovial membrane is uneven: in places of invaginations the synoviocytes are oriented perpendicularly relatively to the surface, in flatten areas the synoviocytes are elongated, longitudinal axis is oriented parallel to the surface of the synovial membrane. The synoviocytes are arranged in 1–2 rows. In control rats, the proliferative activity of cells of the synovial membrane in general and in the transition zone in particular is significantly reduced compared

to the 14th day of life. In rats with experimental undifferentiated dysplasia of connective tissue the proliferative activity of the synovial membrane cells is slightly higher than in control (table. 1) (fig. 3).

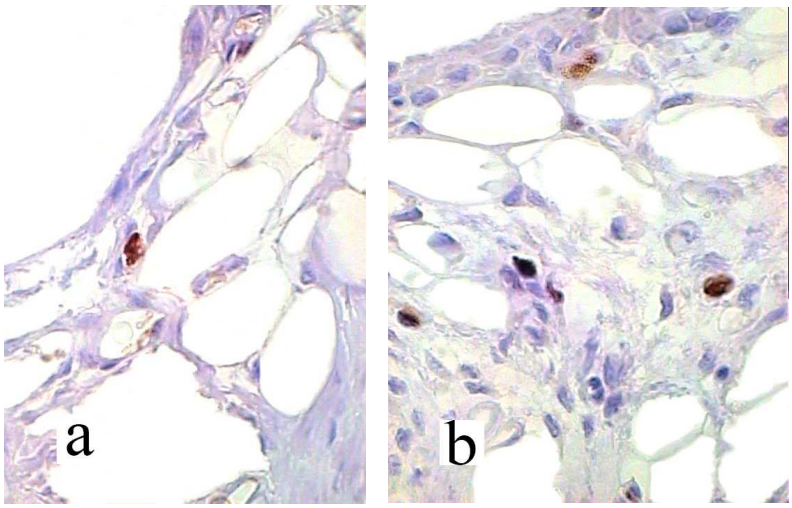


Fig. 3. Ki-67+ cells in synovial membrane of a) the control rat, b) of the experimental rat at the 30th day after birth. x1000.

By the end of the first month of life, the proliferative activity of chondrocytes of all morphofunctional zones of control rats decreases significantly compared to the 14th day of life. In the control rats in a surface zone, ki-67+ chondrocytes are absent; in the intermediate zone there are a few labeled cells (2.5±0.37 %). In rats with experimental undifferentiated dysplasia of connective tissue the proliferative activity of chondrocytes of the superficial (2.82±0.37 %) and intermediate zone (2.50±0.37 %) is maximal in comparison with the control.

In the control rats, subchondral bone is not formed; the articular cartilage is still of embryonic structure. In all morphological zones of the articular cartilage in this group of rats, the relative number of ki-67+ cells is not significantly different.

On the 14th day after birth the level of proliferative activity of synovial membrane cells in control rats is found to increase compared to the 11th day of life. In offspring of rats with experimental undifferentiated dysplasia of connective tissue, the level of proliferative activity of synovial membrane cells is reduced by 2.5 times in comparison with the previous period of observation. In general, the proliferative activity of the synovial membrane cells in control rats shows its maximum, while in rats with experimental undifferentiated dysplasia of connective tissue this index is minimal (table 1). In the transition zone, the highest level of proliferative activity is determined in control rats. This index is significantly higher than in the experimental group of animals (table 1).

Table 1

The relative number of ki-67+ cells in the joint capsule of the knee joint of rats (M±m, %)

	Group of rats	The 1st day after birth	The 11th day after birth	The 14th day after birth	The 30th day after birth
Synovial membrane in general	1	14.00±0.80	3.59±0.37	10.79±0.76	3.00±0.37
	2	0.86±0.40*	0.57±0.40*	3.50±0.37*	4.00±0.37
Transition zone of synovial membrane	1	1.94±0.39	8.21±0.74	6.98±1.14	0.50±0.37
	2	2.57±0.40	0.86±0.40*	2.00±0.37*	1.75±0.37*

Notes: 1 – control rats; 2 – rats with experimental undifferentiated dysplasia of connective tissue; * – significance of the differences in comparison with the control group $P<0.05$

Thus, the proliferative activity of the synovial membrane cells changes dynamically during the first month after birth. On the background of experimental undifferentiated dysplasia of connective tissue, a significant decrease of proliferative activity of the synovial membrane cells during the first two weeks after birth takes place; up to the end of the first month of life this discrepancy is leveled. Along with the low proliferative activity of the synovial membrane cells during the first two weeks after birth in rats with experimental undifferentiated dysplasia of connective tissue significantly lower values of the relative area occupied by fibers on the background of the predominance of the relative area occupied by extracellular matrix of the synovial membrane are determined. Also in newborns with experimental undifferentiated dysplasia of connective tissue a significant increase in the relative area occupied by the cells of the synovium, the increase in the absolute number of mast cells in the conventional unit square and the decrease in the relative area occupied by vessels of the microcirculation are revealed.

Stages of formation of the articular cartilage of rats' femur distal epiphysis are characterized by progressive growth of structure, both through the reproduction of cells, and growth and synthesis of the cartilage matrix. The decrease in proliferative activity may be associated with a lengthening of the mitotic cycle or with an exit of a certain percentage of cells from the mitotic cycle [8].

In newborn rats with experimental undifferentiated dysplasia of connective tissue the highest relative content of ki-67+ cells in the perichondral zone of articular cartilage is defined, this fact is associated with a more rapid rate of differentiation and formation of articular cartilage on the background of experimental undifferentiated dysplasia of connective tissue, due to the emerging of immunological immature lymphocytes from the thymus to the periphery, the functional activity of cells of the transition zone is altered, which is accompanied by accelerated ingrowth of blood vessels and formation of subchondral bone [4].

In rats with experimental undifferentiated dysplasia of connective tissue subchondral bone formed by the 11th day after birth, while in control, articular cartilage acquires the features of a juvenile one by the 14th day after birth [4]. Proliferative activity of chondrocytes of articular cartilage in rats with experimental undifferentiated dysplasia of connective tissue at the 11th day was significantly lower than in control. This result is consistent with the data on the reduced proliferation of chondrocytes, prior to the formation of the secondary center of ossification [5].

By the end of the first month of life, the proliferative activity of chondrocytes in all morphofunctional zones of control rats decreased significantly compared to the 14th day of life.

Conclusions

1. During the first two weeks after birth in rats with experimental undifferentiated dysplasia of connective tissue a proliferative activity of synovial membrane cells is significantly decreased in comparison with control.

2. The distribution of ki-67+ cells in different zones of the articular cartilage of the distal epiphysis of rats' femur during the first month of life is different depending on the degree of morphological and functional differentiation of chondrocytes.

3. In rats with experimental undifferentiated dysplasia of connective tissue the increased proliferative activity of articular cartilage chondrocytes in all morphofunctional zones reduces at the 11th, 14th days after birth, this is associated with earlier formation of subchondral bone (at the 11th day compared to the 14th in control group) and the change in the rate of differentiation and functional activity of chondrocytes.

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Стаття надійшла 24.02.2020 р.