Chapter 2

Chondrocyte Turnover in Lung Cartilage

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Abstract

Cartilage is a highly differentiated connective tissue that forms mechanical support to soft tissues and is important for bone development from fetal period to puberty. It is conformed by chondrocytes and extracellular matrix. It is generally believed that adult cartilage has no capacity to renewal. A delicate balance between cell proliferation and cell death ensures the maintenance of normal tissue morphology and function. Stem cells play essential roles in this process. Mesenchymal stem cells (MSCs) can give rise to multiple lineages including bone, adipose and cartilage. Nestin protein was initially identified as a marker for neural stem cells, but its expression has also been detected in many types of cells, including MSCs. *In vivo*, chondrocyte turnover has been almost exclusively studied in articular cartilage. In this chapter we will review the findings about the chondrocyte turnover in lung cartilage. We have presented evidence that there exist nestin-positive MSCs in healthy adulthood that participates in the turnover of lung cartilage and in lung airway epithelium renewal. These findings may improve our knowledge about the biology of the cartilage and of the stem cells, and could provide new cell candidates for cartilage tissue engineering and for therapy for devastating pulmonary diseases.

Keywords: lung, cartilage, chondrocyte, turnover, apoptosis, proliferation, stem cells

1. Introduction

Cartilage is a connective tissue consisting of cells and extracellular matrix (ECM). These cells are called chondrocytes and reside within spaces called lacunae. The ECM is a three-dimensional

macromolecular network composed of fibers and ground substance. In the mammals, much of the skeleton is first laid down in cartilage, and in the adult body it covers the articular surfaces of bones and forms the sole skeletal support of several structures [1].

Normal chondrocytes maintain a functional ECM that replaces itself very slowly and provides a shock absorber [2]. However, in mature cartilage, metabolic activity is low, and has been thought that adult chondrocytes resist proliferation throughout life. As a result, the mechanical properties of cartilage deteriorate with age [3–5].

Cell death and cell proliferation must be balanced in adult organisms in order to maintain homeostasis. Programmed cell death or apoptosis is important in mature organisms for deleting unwanted cells (e.g. aged cells). Most tissues contain stem cells that are able of proliferate and differentiate to replace cells that have been lost. A defective cell turnover process may have serious consequences to the tissues and the entire organism [6].

The role of chondrocyte turnover in cartilage aging and disease has been poorly analyzed and most of the related studies have been carried out in articular cartilage. In this chapter we will review the findings about the chondrocyte turnover in lung cartilage.

2. Chondrocyte, cartilage, and pulmonary cartilage

2.1. The chondrocyte

There are two forms of cells in cartilage: chondroblasts and chondrocytes. Chondroblasts are actively dividing immature cells which form ECM. They are oval or spindle-shaped cells with a spherical nucleus. The cytoplasm is basophilic, rich in ribosomes, rough endoplasmic reticulum, and Golgi saccules [7].

When chondroblasts are completely surrounded by ECM, they are called chondrocytes. They reside in spaces within the cartilage matrix known as lacunae. However, the cells fill the lacunae *in vivo*, as verified by electron microscopic studies. Chondrocytes vary from elongate to spherical in shape in relation to their position within the cartilage. They have a spherical nucleus with one or more nucleoli. Chondrocyte cytoplasm contains, in addition to glycogen and lipid, the usual characteristics of a secretory cell: abundant rough endoplasmic reticulum and prominent Golgi complex [8, 9].

The main function of the chondrocyte is to produce, maintain, and remodel the ECM of the cartilage. Chondrocytes receive mechanical, electrical, and physicochemical signals transmitted by the ECM and respond by regulating their metabolic activity [3, 9].

2.2. Cartilage

Cartilage is flexible and strong, and is resilient to compression. It forms mechanical support to soft tissues and is important for bone development from fetal period to puberty [1, 10].

Cartilage consists of cells (chondroblasts and chondrocytes) and ECM. The ECM is primarily composed of tissue fluid and macromolecules, including collagens, proteoglycans, and

glycoproteins. Cartilage is subdivided into three varieties depending on their molecular composition: hyaline, elastic, and fibrous [11]. Of these, hyaline cartilage is the most widely distributed type.

With the exception of the free surfaces of articular cartilages, hyaline cartilage is surrounded by a membrane of fibrous connective tissue, the perichondrium. Cartilage is usually devoid of blood vessels, so its cells must obtain their oxygen and nutrients by long-range diffusion from the perichondrium [12].

2.3. Pulmonary cartilage

The upper respiratory tract includes the nose and nasal passages, paranasal sinuses, the pharynx, and the portion of the larynx above the vocal cords. The lower respiratory tract includes the trachea and within the lungs, the bronchi, bronchioles, and alveoli. This system performs or participates in several functions: air conduction, gas exchange, olfaction, and phonation [13–15].

Although the air passages take on their mature appearance well before a fetus is viable, they undergo significant maturational changes in late gestation. Thereafter, the lungs undergo a phase of growth and maturation during the first two decades of live and achieve maximal lung function approximately at the age of 20 years old for women and 25 years old for men. Lung function remains steady from age 20 to 35 years and starts declining thereafter. It has been suggested that airway cartilage plays an important role in determining airway compressibility and distensibility. Age-related differences in airway mechanical function may reflect an increase in stiffness of both airway muscle and cartilage that occurs with increasing age [16–18].

Cartilage (hyaline type) has the function of maintaining airway patency and it also serves for the attachment of local muscle and connective tissue. It exists in the form of plates of cartilage which have characteristics shapes and arrangements at different airway levels [19].

In the trachea and right and left main bronchi, cartilage is present in the anterior and lateral walls as C-shaped plates. Approximately 15–20 cartilaginous rings support the trachea. The cartilage in the wall of intrapulmonary bronchi is in the form of irregular cartilage plates that form a complete but not continuous circumferential support. The smallest bronchi have only widely scattered cartilaginous plates in their walls. Terminal and respiratory bronchioles lack supporting cartilaginous plates [13, 19].

3. Cell turnover

Physiological cell turnover plays an important role in maintaining normal tissue function and morphology. During this process, older differentiated cells are typically eliminated by programmed cell death (apoptosis) and replaced by the division progeny of adult stem cells (ASC) [20, 21].

A delicate balance among all factors influencing cell turnover is needed to maintain the normal volume and function of tissues in healthy people. The key points of this homoeostatic

process are apoptosis and cell proliferation. Cell turnover is precisely regulated by the interplay of various factors, which modulate tissue and cell-specific responses on apoptosis and proliferation, either directly, or by altering expression and function of key death and/or cell proliferative genes [6, 20, 22].

Age-specific changes in tissue regeneration and repair lead to cell loss and compromise of tissue homeostasis, structure, and function. These phenomena parallel changes in resident stem cell function [23, 24].

3.1. Apoptosis

Apoptosis is a process of controlled cellular death whereby the activation of specific death-signaling pathways leads to deletion of cells from tissue [25]. The term apoptosis was first used in a paper by Kerr, Wyllie, and Currie in 1972 to describe a morphologically distinct form of cell death [26], discriminating it from necrosis.

Apoptosis plays an essential role in survival of the organisms and is responsible for many biological processes such as normal cell turnover, embryonic and brain development, proper development and functioning of the immune system, and hormone-dependent atrophy [27, 28].

3.1.1. Apoptosis versus necrosis. Other forms of cell death

Cell death has been broadly classified in two categories: apoptosis and necrosis. Apoptosis is a synchronized and energy-requiring process than involves altered expression of key cell proliferation and death-inducing genes, and the activation of a group of cysteine proteases (caspases) in a complex cascade of events that link the initiating stimuli to the final demise of the cell, while necrosis does not involve gene expression and is a passive externally driven event resulting from acute cellular injury [20, 29]. However, increasing evidence has been accumulating that necrosis can occur in a regulated manner, and that necrosis has a prominent role in multiple physiological and pathological settings [30].

Apoptosis is morphologically characterized by cell shrinkage, detachment from the substrate, chromatin condensation, nuclear and DNA fragmentation, cytoplasmic membrane blebbing, package of the cell debris into apoptotic bodies, and engulfment by resident phagocytes. Necrosis involves increase in cell volume, swelling of organelles, rupture of the plasma membrane, and the subsequent release of the cytoplasmic contents into the surrounding tissue, leading to inflammatory reaction [31].

Recently, new forms of cell death have been progressively described, which can be more precisely distinguished based on molecular pathways. A functional classification of cell death forms have been proposed that includes extrinsic apoptosis, caspase-dependent or -independent intrinsic apoptosis, regulated necrosis, autophagic cell death, and mitotic catastrophe [30, 31].

3.1.2. Apoptosis mechanisms

Apoptosis can be initiated by exogenous stimuli such as ionizing radiation and chemotherapeutic drugs, as well as by endogenous stimuli such as the absence of oxygen, nutrients or growth/survival factors, the presence of DNA damage, or the action of cytokines [32].

There are two main apoptotic pathways: the extrinsic or death receptor pathway, which is triggered from outside of the cell by death ligands, and the intrinsic or mitochondrial pathway, which is triggered from inside the cell as a response to various stress signals. Both intrinsic as well as extrinsic pathways of apoptosis are associated and influence each other [33]. Another pathway of apoptosis as also been recognized that involves T- and NK-cell mediated cytotoxicity and perforin-granzyme-dependent killing of the cell [34].

The three pathways converge on the same execution pathway: the activation of cysteine proteases of the caspase family, which selectively digest the cell from within. The perforin/granzyme pathway also activates another cell death pathway via single stranded DNA damage [29, 34].

3.1.3. Methods of apoptosis detection

Since the pathways of apoptosis are very complicated, there are a lot of features of it than can be evaluated. A great number of methods have been developed to detect apoptosis, such as morphological techniques, proteomic and genomic approaches, spectroscopic methods, flow cytometry, caspase activity assays, microfluidic applications, and electrochemical methods [35]. Each assay has advantages and disadvantages. Understanding the strengths and limitations of the assays would allow investigators to select the best methods for their needs [28, 36]. A description of all assays for detecting apoptosis is beyond the scope of this chapter. We will briefly describe the assays to detect apoptosis most employed by our group.

3.1.3.1. Light microscopy

Detection of apoptotic cells in hematoxylin and eosin-stained tissue sections with light microscopy is possible because of characteristic morphological features of apoptosis. They include condensation of the chromatin in granular masses along the nuclear envelope, cell shrinkage, convolution of the cellular and nuclear outlines, and fragmentation of the nucleus. The apoptotic cell breaks into membrane bound bodies that are quickly removed by neighboring macrophages. The condensed or fragmented nucleus can be detected with DNA dyes such as propidium iodide, Hoechst dye, or DAPI (4′,6-diamidino-2-phenylindole). Light microscopy detects the later events of apoptosis and confirmation with other methods may be necessary [37, 38].

3.1.3.2. Transmission electron microscopy (TEM)

A more definitive method of morphologic identification of apoptotic cells is TEM, because apoptosis is confirmed by several of its ultrastructural characteristics. TEM detects chromatin condensation and convulsions in and around the nuclear envelope that precedes nuclear fragmentation, the condensation of cytoplasm with the disappearance of the microvilli, blebs on the cell surface, and the loss of cell junctions. If immunochemical staining is employed, then chemical information can be also obtained. However, there are limitations in TEM as an apoptosis detection method, including that apoptotic cells detected by TEM are in the last stage of apoptosis, and that much time and a high skill are required for preparation of ultrathin sections used in TEM [35, 39].

3.1.3.3. Terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL)

TUNEL method is based on the assumption that genomic DNA is fragmented in a dying cell, producing fragments of consistent length in apoptotic cell death, as opposed to necrotic cell death where DNA is believed to be randomly degraded [40, 41]. The method consists of the labeling of DNA nick ends by terminal deoxynucleotidyl transferase (TdT) which incorporates the labeled nucleotide (most often dUTP) in the places of DNA strain breaks. The dUTP can then be labeled with a variety of probes to allow detection by light microscopy, fluorescence microscopy, or flow cytometry [42].

TUNEL method is suitable for analysis of apoptosis in individual cells applicable to all kinds of material: cultured cells, tissues, and blood samples, even if a material contains only a few apoptotic cells. Another advantage of the TUNEL staining is that detects cells at a relatively early stage of apoptosis [39, 43]. However, this method also has drawbacks. Notably, it has been reported that the TUNEL assay also detect necrotic and autolytic cells in addition to apoptotic cells [44, 45].

3.2. Cell proliferation

Cell proliferation is the process whereby cells reproduce themselves by growing and then dividing into two equal copies [46]. This process is a fundamental requirement for normal development and homeostasis.

Cell division consists of two consecutive processes, mainly characterized by DNA replication and segregation of replicated chromosomes into two separate cells. The process of replicating DNA and dividing a cell can be described as a series of coordinated events that compose a cell cycle [47, 48].

3.2.1. The cell cycle

The cell cycle can be subdivided into two stages: interphase and mitosis. Genome replication occurs during the interphase, and its segregation to the daughter cells during the mitosis. The interphase includes G_1, S, and G_2 phases. Cells in G_0 are not actively cycling and have to be stimulated by growth factors in order to enter the cell cycle in G_1 [49]. Mitosis includes prophase, prometaphase, metaphase, anaphase, and telophase, and also cell division (cytokinesis), which overlaps the final stages of mitosis [50, 51]. In this chapter we will further analyze only the interphase.

DNA synthesis and doubling of the genome take place during the synthetic or S phase. This is preceded by a period or gap of variable duration called G_1 during which the cell is preparing for DNA synthesis, and is followed by a period known as the second gap or G_2, during which the cell prepares for mitosis [48, 52].

3.2.1.1. Cell cycle regulation

Cell proliferation is a process fundamental to development, growth, homeostasis, adaptation to disease, and neoplasia. For this reason, cell cycle events must be tightly regulated

to ensure that they occur in the correct order with respect to each other and that they occur only once per cell cycle [53].

At least two types of cell cycle regulation mechanisms have been recognized: cell cycle checkpoints, which are surveillance mechanisms that monitor the order, integrity, and fidelity of the major events of the cell cycle [54], and a cascade of activation and deactivation of a series of proteins that relay a cell from one stage to the next [47].

3.2.1.1.1. Cell cycle checkpoints

Cell cycle checkpoints are a series of control systems enabling proliferation only in the presence of stimulatory signals (e.g. growth factors). They also arrest the cell cycle in response to DNA damage in order to provide time for DNA repair. After damage repair, progression through the cell cycle resumes. If the damage cannot be repaired, the cell is eliminated by apoptosis [55].

The primary checkpoint acts late in G_1. Once the cell has entered S phase, it is bound to continue through S, G_2, and M and thus produce two daughter cells. This checkpoint is sometimes known as the "point of no return" in the cell cycle with respect to S phase entry [56].

Additional checkpoints exist in S phase to activate DNA repair mechanisms when necessary. Furthermore, incomplete DNA replication or DNA damage triggers checkpoint pathways that block the G_2/M transition to ensure that cells have completely replicated their DNA and that it is intact before they enter mitosis [57].

Finally, the spindle assembly checkpoint acts during mitosis to maintain genome stability by delaying cell division (cytokinesis) until accurate chromosome segregation can be guaranteed [58].

3.2.1.1.2. Cyclin-dependent kinases (Cdks) regulation

The main families of proteins that play key roles in controlling cell cycle progression are the Cdks, the cyclins, the Cdk inhibitors (CKIs), and the tumor-suppressor gene products—the retinoblastoma protein (pRb) and p53 [59].

Progression of the cell through the cell cycle is mediated by sequential activation and inactivation of Cdks. The Cdks are a family of serine/threonine protein kinases that are activated at specific points of the cell cycle by the cyclins. Cdks activity can be counteracted by cell cycle inhibitory proteins, the CKIs [60, 61].

Activated Cdks induce downstream processes by phosphorylating selected proteins. pRb is a downstream target of Cdks-cyclins complexes [62]. Full pRb hyperphosphorylation releases pRb from E2F relieving repression of E2F target genes and allowing for activated E2F-dependent transcriptional induction and cell cycle progression [63].

p53 is stabilized in response to DNA damage, oncogenic stress, and various other stress conditions and activates transcription of a number of genes (including *p21*, *Mdm2*, and *Bax*) that induce cell cycle arrest or apoptosis. At the G_1/S checkpoint (see above), cell cycle arrest induced by DNA damage is p53-dependent [64].

3.2.2. Identification and measurement of cell proliferation

Assessment of cell proliferation is often of relevance in biomedical science, and a range of techniques have evolved to identify and quantify the process, generally by recognition and calculation of the number of cells in S or M phase [65].

A variety of markers have been used to determine cell cycle status and quantify cell proliferation, including the identification of mitotic figures, tritiated thymidine incorporation, bromodeoxyuridine incorporation, expression of proteins such as the proliferative cell nuclear antigen (PCNA), Ki-67, cyclins and Cdks, and the analysis of Cdks phosphorylation status [62, 66].

Of importance for this chapter are the immunohistochemical methods that detect proliferation-associated antigens. Ideally, such methods should be applicable to routinely processed tissues, they should be relatively inexpensive and the results easily quantified and interpreted [67]. The best known markers employed to recognize proliferating cells are Ki-67 and PCNA.

Cells express Ki-67 during G_1, S, G_2, and M phases, but not during the resting phase G_0. Its levels are low in the G_1 and S phases and rise to their peak level in M. Later in the M phase, a sharp decrease in Ki-67 levels occurs [68]. Ki-67 is required to maintain individual mitotic chromosomes dispersed in the cytoplasm after their release from the nuclear envelope, through a surfactant mechanism [69].

Ki-67 is widely used as a proliferation marker because it provides a rapid and relatively inexpensive method of measuring dividing cells [65, 70]. However, the short half-life of Ki-67 (1–1.5 h, regardless of the cell position in the cell cycle [71, 72]) makes its detection difficult. Furthermore, some healthy tissues can express low levels of Ki-67 [68].

PCNA was first shown to act as a cofactor/auxiliary protein for DNA polymerase δ, which is required for DNA synthesis during replication. However, besides DNA replication, PCNA functions have been associated with other cellular processes such as chromatin remodeling, DNA repair, sister-chromatid cohesion, and cell cycle control [73]. During DNA replication, presence of PCNA is necessary for synthesis of the leading strand. Levels of PCNA expression are therefore highest during S phase, with little to no expression during G_1 and intermediate levels in G_2 and M phases [62, 74].

PCNA detection has been widely used in immunohistochemical studies of cell proliferation. However, some authors claim that PCNA is not a reliable marker of this process because it is a pleiotropic protein involved in several aspects of cell control and not only in proliferation [66]. On the opposite, other authors affirm that PCNA is the most reliable and versatile of all markers used to analyze cell proliferation [75]. In the past, we have successfully used the immunohistochemical detection of PCNA in studies of cell turnover in lung [76].

4. Stem cells

4.1. Definition and classification

Stem cells are generally defined as clonogenic cells capable of both self-renewal and multilineage differentiation [77]. For a cell to be considered a stem cell, it must be capable of

asymmetrical cell division, producing an exact multipotent replica cell, and an additional progeny cell than can perform a more specialized function [78].

Stem cells are classified according to their origin and developmental status in embryonic stem cells (ESC) and adult stem cells. Embryonic stem cells (ESC) can be derived from the inner cell mass of a blastocyst during gastrulation. They are totipotent cells giving rise to the germ line during development and virtually to all tissues of the organism [78, 79]. Adult stem cells (ASC) are tissue-resident stem cells that, based on their differentiation potency, can be classified as multipotent, oligopotent, or even unipotent [80]. In their tissue of residency, ASC function as lineage-committed progenitors to cells capable of more highly specialized tasks [78]. They are involved in tissue homeostasis and repair after wounding over the lifetime [79].

Among the tissues and organs harboring ASC, there are bone marrow, vascular walls, adipose tissues, skeletal muscles, heart, and brain, as well as epithelium of lung, liver, pancreas, digestive tract, skin, retina, breast, ovaries, prostate, and testis [81]. The bone marrow stem cell niche includes the hematopoietic stem cell population, which provides continuous renewal of blood cell lineages and the foundation of the immune system, and the mesenchymal stem cell population, responsible for osteogenic, adipogenic, and chondrogenic differentiation [82].

4.2. Mesenchymal stem cells (MSCs)

The minimal criteria for defining MSCs include: (a) remain plastic-adherent under standard culture conditions; (b) express CD73, CD90, and CD105, and lack expression of CD34, CD45, CD14 or CD11b, CD79a or CD19, and HLA-DR surface molecules; and (c) differentiate into osteoblasts, adipocytes, and chondrocytes *in vitro* [83, 84].

Originally isolated from bone marrow, MSCs have being isolated from other sites including spleen, thymus, muscle, adipose tissue, endometrium, placenta, umbilical cord, umbilical cord blood, peripheral blood, periosteum, periodontal ligament, dental pulp, synovium, synovial fluid, tendons, and cartilage [84, 85]. A perivascular location for MSCs has been suggested, correlating these cells with pericytes. This would explain why MSCs can be virtually isolated from all tissues [79, 86].

MSCs have demonstrated significant potential for clinical use due to their convenient isolation, their lack of significant immunogenicity permitting allogenic transplantation, their lack of ethical controversy, and their potential to differentiate into tissue-specific cell types [87]. MSCs may have therapeutic applications in several clinical disorders including myocardial infarction, diabetes, sepsis, hepatic failure, acute renal failure, several kinds of lung disease, as well as in spinal cord injuries, and bone and cartilage diseases [88, 89].

4.3. Nestin-positive MSCs

The human nestin protein consists of 1621 amino acids and displays a predicted molecular weight of 177.4 kDa. It is a class VI intermediate filament protein. Intermediate filaments represent, along with microtubules and actin filaments, one of the main components of cytoskeleton in animal cells [90].

Although nestin was first described as a marker of neural stem cells [91], its expression has also been shown in various prenatal and adult cells and tissues. Nestin-expressing cell types in embryonic and fetal tissues includes developing skeletal muscle cells, developing cardiomyocytes, endothelial cells of developing blood vessels, pancreatic epithelial progenitor cells, and hepatic oval cells. In adult, nestin expression has been found in, for example, satellite cells in dorsal root ganglia, retina, pancreatic stellate and endothelial cells, interstitial cells of Cajal, muscularis propria, Sertolli cells, and odontoblasts. Nestin has also been found to be expressed in injured tissues and in cancer cells [92].

In most of the studies, nestin has been detected by immunohistochemistry [92]. The principal advantage of immunohistochemistry over other techniques is that it enables the observation of processes in the context of intact tissue [93].

Normally, nestin becomes up-regulated in tissues during embryogenesis and down-regulated during maturation. During tissue injury in the adult, nestin is expressed in cells with progenitor cell properties. Furthermore, observational and interventional studies in animals and humans have shown that nestin may be an important marker for MSCs. These cells seem to act as a tissue reserve and to participate in tissue repair, regeneration, and growth [94, 95].

5. Cell renewal in lung cartilage

Cartilage grows by two methods: appositional growth and interstitial growth. In the former, chondroblasts in the perichondrium are transformed into chondrocytes. Interstitial growth result from mitotic division of pre-existing chondrocytes within the matrix. These two mechanisms occur early in life [96].

In the past, it has been believed that healthy adult chondrocytes maintain a stable resting phenotype and resist proliferation and differentiation throughout life [5]. Most cell types reach cell cycle arrest after a characteristic number of population doublings. The limit for human chondrocytes has been estimated at ~35 population doublings [4]. Their decreasing proliferative potential has been attributed to replicative senescence associated with erosion of telomere length [97].

We analyzed lung specimens from adult mice embedded in paraffin. Apoptosis was analyzed by TUNEL assay. PCNA and nestin were examined by immunohistochemistry. Apoptosis and PCNA were detected in lung chondrocytes. Serial section analysis demonstrated that cells in apoptosis were different from PCNA-positive cells, indicating that turnover was occurring. Chondrocytes were negative for nestin. However, nestin-positive cells were found in connective tissue associated with cartilage, in some specimens in close proximity of it and in perivascular cells. Thus, the findings of this work indicated that cell turnover in adult lung cartilage is possible, and that it may be mediated by nestin-positive cells [98].

In another related work, we found nestin-positive cells inside of lung cartilage and cells in division very close from them. This finding indicated that there exist nestin-positive MSCs in the adult that are able to differentiate into lung chondrocytes, perhaps to maintain homeostasis and/or repair damaged tissue [99].

For a long time it has been considered that cartilage contains a unique type of cell: the chondrocyte. However, nestin-positive MSCs has been found in cultured human adult lung cells, which underwent chondrogenic differentiation [100], and evidence from our investigations [98, 99] indicates that besides chondrocytes there exist nestin-positive MSCs in the adult lung cartilage.

The nestin-positive MSCs might be circulating in the blood stream or remain located in local blood vessels and be able to populate the cartilage when necessary, and/or might reside inside it. Other authors have shown that murine MSCs embolised within pulmonary blood vessels following systemic injection, and then transmigrated and differentiated into cartilage [101].

Finally, in another work, we found nestin-positive cells in perivascular areas and in connective tissue that were in close proximity of the bronchial airway epithelium. Nestin-positive cells were also found among the cells lining the airway epithelium, perhaps in order to participate in epithelial renewal [102]. Thus, stem cell reported in our works might be a pluripotent cell, which are able to generate several types of lung tissues. Other researchers presented evidence that a pluripotent stem cell exists in the lung that can generate lung-like tissue *in vitro* [103, 104].

6. Conclusion

Most of cells, tissues, and organs show continuous turnover. A delicate balance between cell proliferation and cell death ensures the maintenance of normal tissue morphology and function. Stem cells play essential roles in the growth, homeostasis and repair of many tissues. MSCs can give rise to multiple lineages including bone, adipose, and cartilage. The intermediate filament protein nestin was initially identified as a marker for neural stem cells, but its expression has also been detected in many types of cells, including MSCs.

It is generally believed that adult cartilage has no capacity to renewal. Taken together, our findings indicate that there exist nestin-positive MSCs in healthy adulthood that participates in the turnover of lung cartilage and in lung airway epithelium renewal. These findings may improve our knowledge about the biology of the cartilage and of the stem cells, and could provide new cell candidates for cartilage tissue engineering and for therapy for devastating pulmonary diseases.

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