Original Article

New Developments in Understanding the Histological Structure of Human Ear Cartilage

(auricular cartilage / layered arrangement of chondrocytes / immunophenotype of chondrocytes/ maintenance of fixed shape of the pinna)

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Abstract. Histological, immunohistochemical and molecular examination of bioptic samples of 30 normal adult auricular cartilages and small samples from 6 ear cartilages from aborted foetuses was performed. The adult cartilage was the tissue with minimal proliferative activity, which we were able to confirm with antibodies against Ki67 in contrast to a high proliferative activity in the auricular cartilage of foetal tissues. It may therefore be presumed that the process of foetal tissue maturation is undoubtedly associated with a significant reduction in proliferative activity. The mature lamella of the adult auricular cartilage has a histological tri-lamellar structure. There are a great number of elastic fibres in the intercellular matrix of the central zone, which are conversely present in only small amounts in both peripheral layers. While the external layer of the concave surface of the cartilage contains a fewer number of oval elements, the external layer of the convex side is composed of numerous fusiform chondrocytes. Antibodies against various subtypes of S-100 protein showed that auricular chondrocyte activity is modified depending on the configuration of individual distinct zones (isoforms A1, A6, B2 and P were positive in all layers, isoforms A2 and A2 in peripheral zones). The most active cells metabolically are most likely chondrocytes in both external layers adjacent to the perichondrium. We have also demonstrated α-smooth muscle actin (SMA)-positive chondrocytes in both peripheral layers of the auricular cartilage adjacent to the perichondrium. In addition, we found definite differences in the distribution of actin-positive cells depending on the external shape of the pinna. The majority of these fusiform cells were localised primarily in the areas of great curvature of the pinna, especially the convex side, as mentioned above. On the basis of these unique structural features we assume that the ear cartilage may embody an example of the so-called intelligent biological material, which has its internal structure made in such a way as to more easily develop and yet still maintain all the shape characteristics of the human auricle. The knowledge of these specific structural characteristics is important especially for use of auricular cartilage in auricular reconstruction.

Introduction

The human auricle, with its characteristic external appearance, is a well-known structure of the head, and is composed of soft tissues and elastic cartilage. Its gross configuration is to a certain extent species specific: it has quite distinct characteristic features in humans, and differences between individuals are relatively small. The shape of the human ear is undeniably genetically determined, but its formation would not be possible without characteristic properties of individual tissue components from which it arises, and which ensure soundness of structure for the entire life of the individual.

It is therefore surprising that only minimal attention has been focused on the constitution of individual components of this complex structure (Sternberg, 1997; Hy-

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Abbreviations: ABC – avidin-biotin complex, SMA – smooth muscle actin.

bášek, 1999; Gnepp, 2009; Griffin et al., 2016; Chiu et al., 2017). During this research, a surprising dearth of information was found in the scientific literature on this dilemma. Griffin et al. (2016) concluded in their histological study that auricle has a homogenous structure in term of chondrocyte morphology, extracellular matrix and elastin content. In their study only standard staining methods for glycosaminoglycans, proteoglycans, elastin and collagen type I were used and specific microstructural organization of the auricular cartilage was not recognized. However, this study provides new information on the comprehensive biomechanical properties. Chiu et al. (2017) compared the microstructural, biochemical and mechanical properties of auricular cartilage from different species and described some differences in the size of chondrocyte lacunae. Human auricular cartilage had similar biochemical composition to both rat and rabbit.

In the course of our study, we, however, discovered that some structural characteristics of the human auricular cartilage are to a certain extent unique when compared to the histological structure and functional attributes of other cartilaginous tissues, especially articular cartilage. On the basis of the result of histological and immunohistochemical examination we demonstrate here that auricular cartilage has rather non-homogenous and specific histological structure. We therefore consider it useful to summarize our histological and immunohistochemical findings, which may contribute to substantiating the general perceptions of the microscopic structure and functional characteristics of the human ear cartilage. The knowledge of these histologic characteristics is very important because autologous cartilage provides the gold standard for auricular reconstruction (Becker et al., 2003; Araco et al., 2006; Griffin et al., 2016) and we believe that they can also affect the mechanical properties of the auricular implants used during auricular reconstruction.

Material and Methods

Thirty samples of auricular cartilage for inclusion in our study were obtained during elective surgeries performed in the ENT department of General University Hospital in Prague and during routine bioptic examination at the Institute of Pathology of the First Faculty of Medicine of Charles University. In addition, we examined portions of ear cartilages from six aborted foetuses during postmortems performed at the aforementioned department. This study was approved by the Ethical Committee of the General University Hospital in Prague. Each patient received a full explanation on the purpose of this study and submitted an informed consent.

Histological examination

Samples of auricular cartilage obtained during surgery for therapeutic or reconstructive procedures were processed by standard procedures for histological examination. After fixation in 10% formalin solution and tissue dehydration, the samples were processed with paraffin and sectioned into 5-micron sections. Each section was stained with haematoxylin and eosin to evaluate the general morphology and cell organization. Staining with alcian blue allowed evaluating the content of proteoglycan. Masson trichrome technique combined with elastic staining was used for demonstration of elastic fibres (Bancroft and Gamble, 2002)

Immunohistochemical examination

For the purposes of immunohistochemical studies, we used the avidin-biotin complex (ABC) technique. All samples were examined using antibodies against α-SMA (monoclonal, 1A4 clone, 1 : 100, Dako, Glostrup, Denmark) and muscle-specific actin (monoclonal, HHF35 clone, 1 : 100, Dako), which is an antibody reacting with all three isotypes of α-actin (skeletal, cardiac, and smooth muscle). In addition, we also performed examination with other antibodies in individually analysed groups, as described below.

Specifically, we used antibodies against: desmin (1 : 200, Dako); h-caldesmon (1 : 50, Dako); vimentin (1 : 300, Bio-Genex, San Ramon, CA); CD68 (KP1 clone, 1 : 20, Dako), myogenin (1 : 50, Dako), myoD1 (1 : 50, Dako). Furthermore, we used antibodies against GFAP (glial fibrillar protein, 1 : 200, Dako) and polyclonal antibodies against S-100 protein (1 : 1,600, Dako) and its various isoforms. The antibodies against S-100 protein isoforms (Neo Markers, Fremont, CA) were diluted in the following ratios: S-100 A1 (1 : 25); S-100 A2 (1 : 50); S-100 A4 (1 : 200); and S-100 A6 (1 : 200). Antibodies against S-100 P proteins (5 mg/ml) and S-100 A10 (5 mg/ml) were supplied by R&D Systems (Minneapolis, MN).

The proliferative activity of auricular cartilage chondrocytes was tested using antibodies to Ki67 (MIB clone, 1 : 50, Dako). The selected samples of human auricular and articular cartilage were examined with antibodies against CD34 (1 : 200, Immunotech S.A., Marseille, France).

RT-PCR analysis

In selected cases involving non-tumorous cartilaginous tissues, RT-PCR analysis was used with the help of primers against all six known isoforms of actin and desmin (Table 1). We used deparaffinized samples comprising only cartilaginous tissues. In total, we analysed 17 samples using this method, five from the elastic cartilage of the pinna.

Isolation of the total RNA, synthesis of cDNA, and RT-PCR analysis were all done using standard operating procedures (Kaňa et al., 2006). The protocol conditions (details in Tvrdík et al., 2005) were as follows: denaturation at 95 °C for 3 min; annealing at 57 °C for 1 min; and extension at 72 °C for 1 min. This was repeated for a total of 45 cycles under the following conditions: denaturation at 95 °C for 1 min; annealing at 45–57 °C for 1 min; and extension at 72 °C for 1 min. The entire reaction was terminated with a final extension lasting for a total of 10 min at 72 °C.

Table 1. List of primers used for RT-PCR analysis

Gene	Primer	Sequence (5´ to 3´)	T_a (°C)	PCR product (bp)
α-SKA	sense	TTCCATTTTCTTCCACAGGG	53	103
α-SKA	antisense	GCTGCCATCGTAAACTGACA	53	103
α-SMA	sense	AGGTAACGAGTCAGAGCTTTGGC	53	199
α-SMA	antisense	CTCTCTGTCCACCTTCCAGCAG	53	199
α-CAA	sense	CCTTCTCTCTCCATCTACCTTCC	47	177
α-CAA	antisense	AGGTTGCAAGTCCTAGTCTGG	47	177
γ-SMA	sense	CCACCTTCCAGCAGATGTG	47	155
γ-SMA	antisense	AGGCTTGTAGGTTTTAATGTTTCA	47	155
α-Actin	sense	AGGCCAACCGCGAGAAGATGACC	53	332
α-Actin	antisense	GAAGTCCAGGGCGACGTAGCAC	53	332
γ-Actin	sense	GTCTGTGGCTTGGTGAGTCT	57	166
γ-Actin	antisense	GAAACTGGGTCCTACGGCTT	57	166
Desmin	sense	TCAGCTTCAGGAACAGCAGG	57	224
Desmin	antisense	GGTGTCGGTATTCCATCATC	57	224

T_a = annealing temperature; bp = base pairs; α-SKA = α-skeletal actin; α-SMA = α-smooth muscle actin; α-CAA = α-cardiac actin; γ-SMA = γ-smooth muscle actin

Results

Findings on auricular cartilages from aborted foetuses after postmortem

Histological examination of tissue samples from aborted foetal auricular cartilages demonstrated that the auricular cartilage base is composed of immature mesenchymal cells, which in contrast to adult elastic cartilage do not express either the S-100 protein or the smooth muscle actin (SMA). Preliminary production of elastic fibres was demonstrated only in certain areas of the auricular cartilage in sections stained for elastic fibres (Fig. 1A). Another fundamental difference to adult auricular cartilage was the finding of high proliferative activity of chondrocytes. This was confirmed using antibodies to Ki67 (Fig. 1B). Nuclear positivity was demonstrated using this antibody in 20 % of cells.

Results in normal auricular cartilage from adult patients

Normal auricular cartilage of the elastic type was found in all investigated cases at the relevant localization. Chondrocytes reside in lacunae; these are surrounded by an interstitial matrix rich in proteoglycans, which is readily stained by reactions to demonstrate mucopolysaccharide acids, namely, alcian blue. Only rarely were the lacunar spaces empty. In addition to the non-fibrillar components of the interstitial matrix, there are multiple elastic fibres, which form a thick lattice around the individual chondrocytes. This was well defined after histochemical reaction for staining elastic fibres (Fig. 2A and 2B).

Upon analysis of histological slides using staining to detect elastic fibres, we found that the cartilage structure from the central lamellae of the auricular cartilage dif-

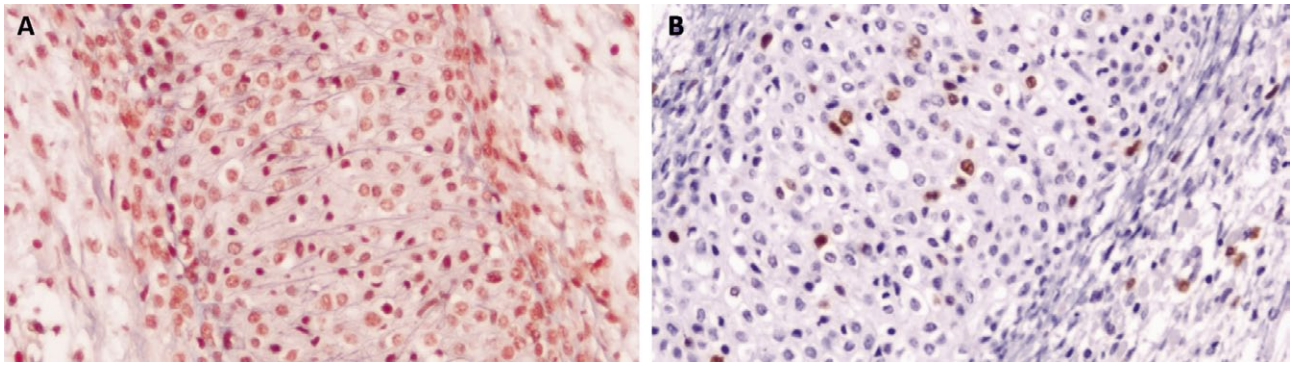

Fig. 1. (**A**) Biopsy sample of ear cartilage from an aborted foetus (22 weeks)
Auricular cartilage was composed of immature mesenchymal cells with rare elastic fibres. Masson trichrome technique combined with elastic staining. Magnification 200×. (**B**) Foetal auricular cartilage (22 weeks) showing high proliferative activity of immature chondrocytes in immunohistochemical reaction with antibody against Ki67. Nuclei of proliferating cells are brown. Magnification 200×

fered from that in the periphery in that there was greater concentration of elastic fibres. This finding implied that the cartilage of the pinna is comprised of three layers of different thickness, which vary in the concentration of elastic fibres (Fig. 2A and 2B).

The density of chondrocytes, as well as the concentration and shape of chondrocytes, was different based on whether they were located on the external surface in an area of concavities (scapha, fossa antihelicis), or on the external surface of the convex side (helix, eminentia scaphae, anthelix). The external surface of the concave side was less cellular; the chondrocytes were oval-shaped, and there were more elastic fibres interspersed between individual cells. On the other hand, the external surface of the convex side contained greater concentration of cells, which had a more fusiform and elongated shape, and they were surrounded by a smaller number of elastic fibres. The most evident difference in the structure of the external surfaces was shown upon reaction with antibody against the S-100 protein (Fig. 2C and 2D). In areas outside of the curves, the assemblage of both peripheral zones was more or less identical.

Analysis of expression of various isoforms of the S-100 protein showed positivity in all layers of auricular cartilage in certain cases. Specifically, this concerned the S-100 protein confirmed with the help of polyclonal antibodies (Fig. 2C and 2D) and its A1, A6, B2, and P isoforms (Fig. 3A). In contrast to monoclonal antibodies

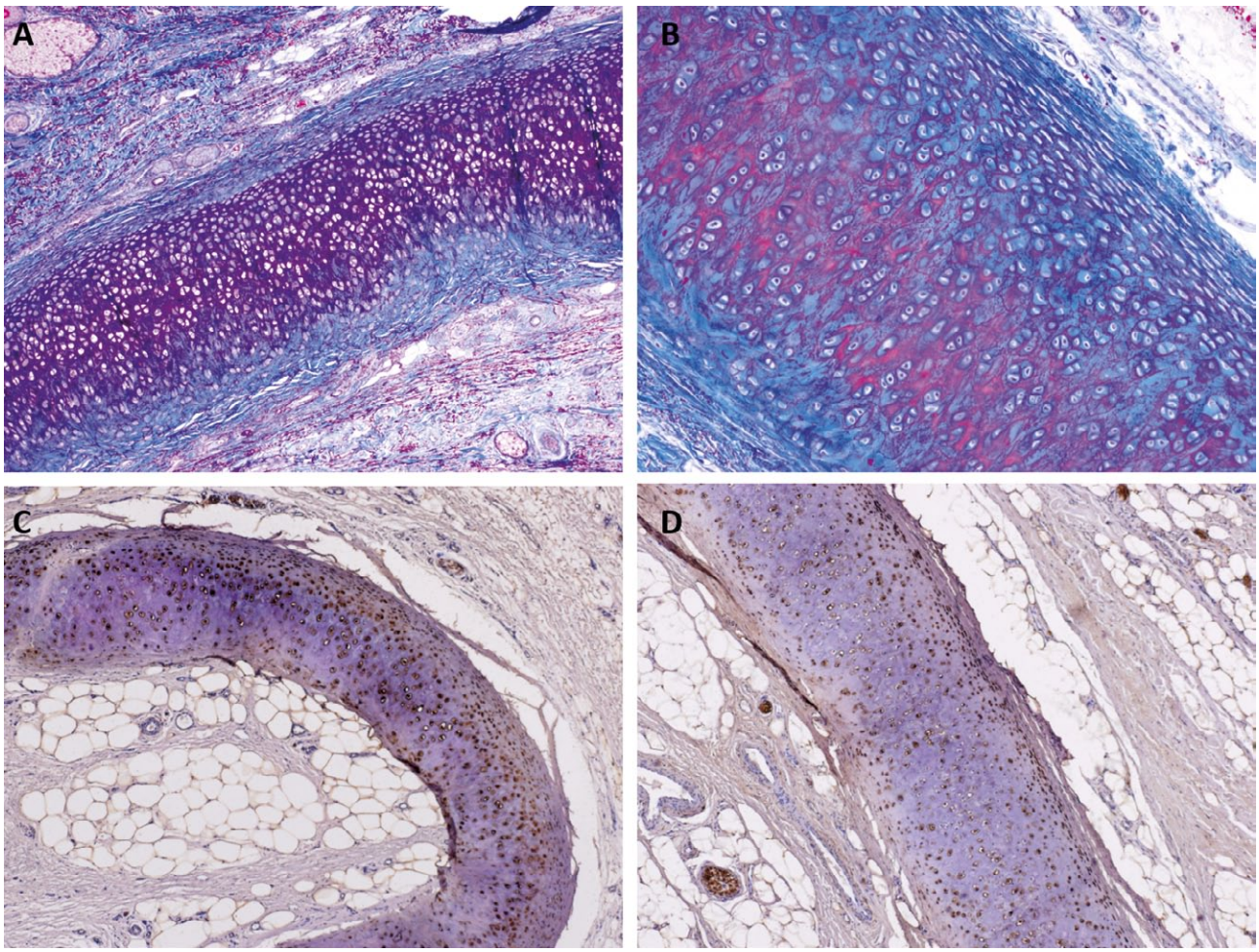

Fig. 2. (**A**) Histological section of adult auricular cartilage showing tri-lamellar structure
In the central part of the cartilage lamellae, there is greater concentration of elastic fibres (in violet colour) in contrast to both peripheral external parts. Masson trichrome technique combined with elastic staining. Magnification 40×. (**B**) Detail from the previous picture showing greater concentration of fusiform chondrocytes in the external surface of the convex side of auricular cartilage. Masson trichrome technique combined with elastic staining. Magnification 200×. (**C**) Positive expression of S-100 protein in adult ear cartilage chondrocytes was predominantly observed in both peripheral zones adjacent to the perichondrium. The density and shape of actin-positive chondrocytes differed based on whether they were located in the area of concavities or on the external surface of the convex side. The external surface of the concave side was less cellular and the chondrocytes were oval-shaped. The external surface of the convex side contained greater concentration of cells with a more fusiform and elongated shape. Magnification 40×. (**D**) Detail from the previous picture showing differing density and shape of S-100 protein-positive chondrocytes in the external and internal surfaces of the convex sides of auricular cartilage. Magnification 100×

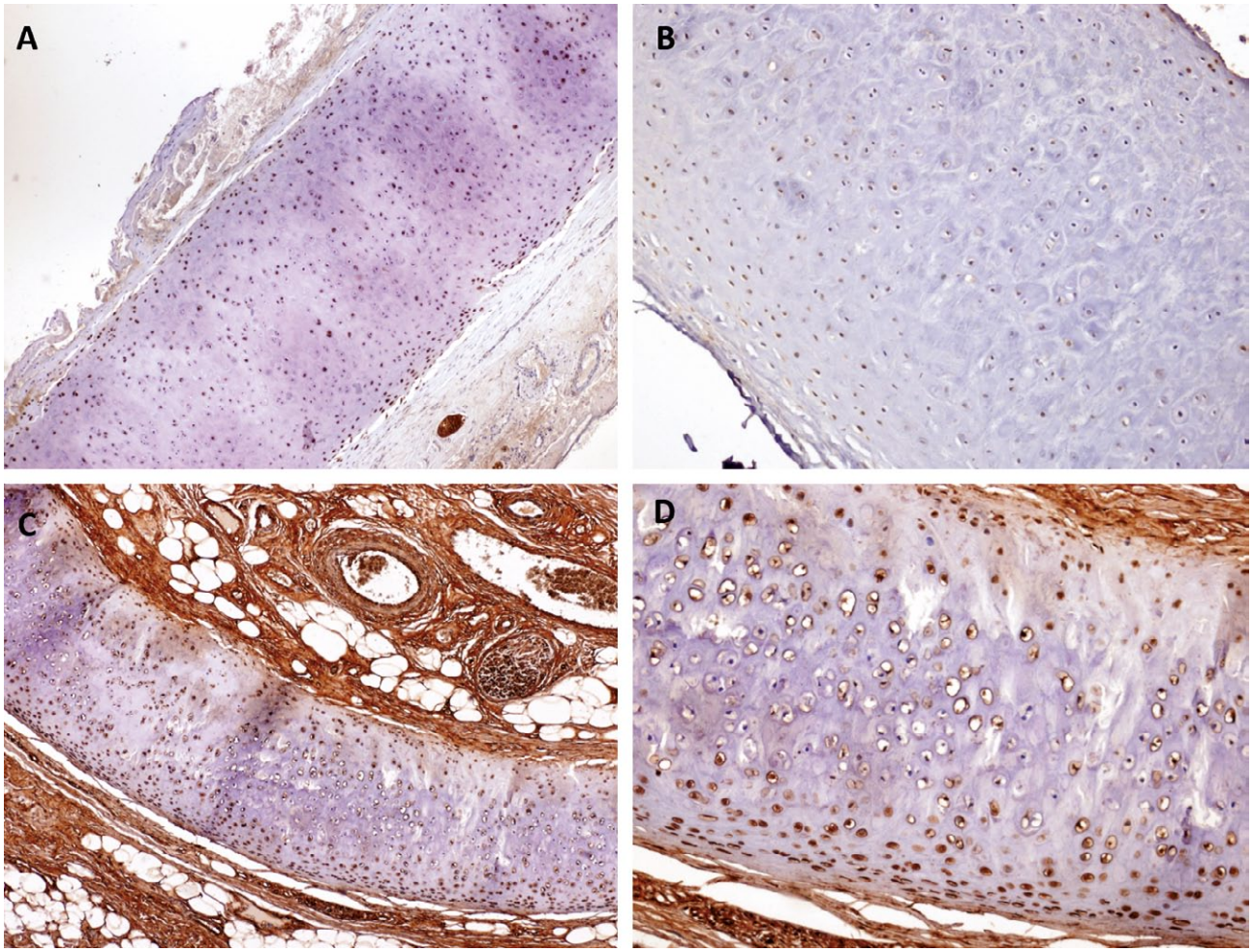

Fig. 3. (**A**) Immunohistochemical reactions with antibody against S-100 A6 protein was positive in all three layers of the ear cartilage. Magnification 100×. (**B**) S-100 A2 protein was predominantly positive in the peripheral zones of the ear cartilage. × 200. (**C**) α-SMA-positive chondrocytes are the dominant type in the adult elastic cartilage. The chondrocytes with α-SMA positivity were predominantly found in the peripheral areas of the auricular cartilage. Magnification 100×. (**D**) Detail from the previous picture showing differing density and shape of α-SMA-positive chondrocytes in the external and internal surface of the convex sides of auricular cartilage. Magnification 200×

against A2 and A10 S-100 protein isoforms (Fig. 3B), the results were positive predominantly in chondrocytes of both peripheral zones found adjacent to the perichondrium. Antibodies against the A4 S-100 protein showed practically no positive results, or the positivity was very slight.

After immunohistochemical examination of normal auricular cartilage, in addition to demonstrating S-100 protein positivity (Fig. 2C and 2D), it was observed that there was an as yet undescribed positivity of α-SMA in the majority of chondrocytes *(*Kaňa et al., 2006*)*. This actin positivity of chondrocytes (Fig. 3C and 3D) was observed in approximately 60 % of cellular population of the auricular cartilage. The chondrocytes with actin positivity were predominantly found in the peripheral areas, whereas the central zones primarily contained actin-negative chondrocytes (Fig. 3C and 3D). These cells exceptionally reacted positively with the antibody for muscle-specific actin. The results of reactions to prove the presence of h-caldesmon and desmin were, however, negative.

A further remarkable and as yet undescribed finding was the occurrence of CD34-positive cells in the perichondrium. These cells were present in only 20 % of all examined samples. In addition, this comprised only individual cells whose distribution was entirely irregular. Their similarity to chondrocytes was confirmed by a positive reaction against the S-100 protein antibody.

We rarely found positivity for several chondrocytes in reactions with the antibodies against GFAP. The proliferative activity of the chondrocytes was completely negative, as demonstrated using antibodies against Ki67.

RT-PCR analysis (Kaňa et al., 2006) confirmed the presence of α-SMA mRNA in the auricular chondrocytes in all five tested samples, and the results were consistent with those of immunohistochemical examination. The presence of mRNA for other tissue-specific isoforms of actin (α-SKA, α-CAA and γ-SMA) was not

demonstrated (Table 1). Reactions for detecting mRNA of desmin and CD 34 positivity were also abrogating, which was due to the particular choice of samples. We were, however, able to confirm the presence of mRNA for β- and γ-actin, which are inherent in the usual non-specific moiety of the cellular cytoskeleton.

Discussion

In addition to the auricular cartilage, elastic cartilage is present in several smaller areas such as the cartilaginous portion of the external acoustic meatus, the wall of the Eustachian tube, and certain areas of the laryngeal cartilage (epiglottis, cartilago cuneiformis et corinuculata, processus vocalis cart. arytenoideae). It also sometimes cooperates in the formation of the small bronchus skeleton. When compared with other types of cartilage, this subtype is differentiated primarily in the density of elastic fibres. This fact has been well known for a number of years. The histological and immunohistochemical examination of non-transformed or normal auricular cartilage has, however, revealed newer findings which, according to our knowledge, have not yet been discussed in the scientific literature (Sternberg, 1997; Hybášek, 1999; Gnepp, 2009; Griffin et al., 2016; Chiu et al., 2017). Most importantly, it has been shown that the superficial layers of auricular cartilage differ in certain regions from other cartilage types not only by the lower density of elastic fibres, which was ascertained only in tissue blocks stained with methods to detect elastin, but also by the shape and density of chondrocytes. At the convexities, the outer layer of auricular cartilage displayed smaller concentration of elastic fibres, which enveloped only the individual chondrocytes. These were, however, more numerous, with an elongated and spindle-shaped form. In contrast, the external layer of the concave surface contained fewer chondrocytes with an oval shape, and there were more elastic fibres between them.

Ki67 antibodies recognize a 395-kDa nuclear antigen, the expression of which is confined to late G1, S, M and G2 growth phases of the cell cycle. They can be used as a measure of the growth fraction in the cell tissue population (Dabbs, 2006). The reaction to confirm the presence of Ki67 was, in adult auricular cartilages, completely negative, in contrast to that of auricular cartilage in premature foetal postmortems. At the same time, this finding demonstrates that with ascending age, in the phase of elastic cartilage maturation, the cell cycle of auricular chondrocytes is apparently prolonged to a significant extent. The auricular cartilage is therefore no different from other cartilage tissue types in its extremely low proliferative activity, which cannot be monitored using contemporary immunohistochemical reaction methods, namely Ki67 examination. We were, however, able to demonstrate that in the case of foetal tissues that were aborted around the 22nd week of gestation, the proliferative activity was remarkable, and it involved approximately 20 % of cells. With foetal growth, and primarily with maturation into adulthood, the proliferative ability is significantly limited, which we regrettably could not demonstrate due to the absence of relevant material.

In the process of immunohistochemical analysis of normal samples of auricular hyaline cartilage, we were the first to attempt to more closely specify the expression of individual isoforms of the S-100 proteins. Polyclonal antibodies against the S-100 proteins are, except for melanocytic lesions, neuroectoderm tumours, and myoepithelium, mostly utilised for chondrocyte identification (Weiss and Dorfman, 1986; Donato, 1999; Cross et al., 2005; Povýšil et al., 2008a). This protein is present in chondrocytes of both non-tumorous and tumorous origin. According to the producer (DAKO), the S-100 protein also reacts with adipocytes, reticular cells of lymph nodes, and glial cells. It is an admixture of antibodies against three subspecies of S-100 protein of the bovine brain, specifically, against S-100 proteins A1, A6 and S-100 B. The specificity of commonly used antibodies against this protein is to a certain extent limited. This must be taken into consideration during the interpretation of results from immunohistochemistry, as described in one of our previous studies (Povýšil et al., 2008a). This was the principal reason why we used antibodies reacting against other known subspecies of this protein.

Currently, the S-100 protein comprises a group of 19 low molecular proteins that bind Ca^{2+} and are expressed on a series of various cell types, among others, chondrocytes (Weiss and Dorfman, 1986; Donato, 1999; Cross et al., 2005; Povýšil et al., 2008a). They participate in the regulation of a number of intracellular processes such as protein phosphorylation, enzyme activation, cellular proliferation, differentiation, structural organization of the cytoskeleton and the cellular membrane, and intracellular homeostasis of calcium. In addition, certain isoforms of the S-100 proteins are sequestered into the extracellular space and assist with chemoattraction of leukocytes, cell proliferation modulation, and regulation of macrophage activity. It has been demonstrated (Donato, 1999; Cross et al., 2005; Povýšil et al., 2008a) that some S-100 proteins influence tumour growth, e.g., by mediation of tumour suppressor genes. In this situation it is quite likely that in the majority of cases, the S-100 protein positivity is an expression of cellular activity, which at present cannot be properly specified.

While the majority of chondrocytes react with polyclonal antibodies, it has been shown that using the available antibodies against various subtypes of S-100 proteins, the results differ to a certain extent, because the chondrocyte activity is modified (by a yet unknown mechanism) depending on the configuration of individual distinct zones of the auricular cartilage. Antibodies against polyclonal S-100 proteins, S-100 A1, A6, B2 and P, displayed strong positivity to chondrocytes in all layers of the auricular cartilage. The reaction to antibodies against the A4 S-100 protein was minimal in all layers. Antibodies against S-100 A2 and A10 displayed activity only in the peripheral layers of auricular carti-

lage, whereas the reaction in the central portion was negative or minimal. These findings may support the supposition that the most metabolically active cells are chondrocytes in both external layers of the adjacent perichondrium.

In our study (Kaňa et al., 2006), we were the first to inform on the presence of actin-positive chondrocytes, which comprise up to 60 % or the entire cell population of normal auricular cartilage and are predominantly found in the superficial layers of normal auricular cartilage (Kaňa et al., 2006). Actin-positive cells, which in identical samples also had the relevant mRNA proven by RT-PCR, had the appearance of typical chondrocytes.

Immunohistochemical examination of the auricular cartilage, similar to the study of articular cartilage in normal and various pathological conditions, has once again opened the question of the presence and significance of α-SMA in chondrocytes of various cartilaginous tissues (Povýšil et al., 1997). Povýšil et al. (1997) were the first to describe this with the help of immunohistochemical and electron microscope examination of actin filaments in the cytoplasm of cells from a chondroblastoma. The authors proposed the term myochondroblasts and myochondrocytes for this finding. Since then, greater attention has been paid to this problem in the scientific literature. For example, the expression of α-SMA was described in the chondroid cells of canine meniscus (Mueller et al. 1999); in the healing of defects in canine articular cartilage (Wang et al. 2000); in the intervertebral discs (Schneider et al. 1999); and in the chondrocytes of human cartilage with osteoarthritic changes (Kim and Spector, 2000; Spector, 2001).

Our study has unequivocally shown that in normal conditions, actin-positive chondrocytes are the dominant type in auricular cartilage (Kaňa et al. 2006), while these cells are normally found in the superficial layers only (Povýšil et al., 2008b). Proliferation of these cells occurs predominantly in various pathological conditions, including conditions after transplantation of a culture of articular chondrocytes (Povýšil et al., 2008b; Horák et al., 2014). Considering this, it is appropriate to briefly recapitulate the findings on various types of actin, as they are known at present.

Actins are ubiquitous eukaryotic proteins found not only in muscle cells, but also in other cells of various histogenesis. They assist in various cellular functions including muscle contraction, cell motility, cellular transport, intercellular adhesion, phagocytosis, mitosis, and maintenance of cell shape and integrity (Vandekerckhove et Weber, 1981; Ramaekers et Bosman, 2004). Approximately half of the cytoplasmic actin in non-muscle cells is found in actin monomers, which represent the globular protein associated with ATP (G-actin). The second half is polymerized in the form of actin filaments (F-actin), which are readily identifiable under electron microscopy.

With the help of electrophoresis, it is possible in vertebrates to differentiate six isoforms of actin, which are divided into two classes (Vandekerckhove and Weber, 1981). The first class includes: cytoplasmic β-actin, cytoplasmic γ-actin, and γ-SMA. Cytoplasmic β-actin and γ-actin are ubiquitous. The subsequent four isoforms of actin are tissue-specific and are present in the skeletal muscle (α-skeletal actin), cardial muscle (α-cardial actin, α-skeletal actin), and in vascular and gastrointestinal muscle (α- and γ-SMA).

α- and γ-SMA are typically encountered in smooth muscle cells, and their principal ability is that of contraction. In the smooth muscle of vessels α-SMA predominates, whereas in the smooth muscle of the gastrointestinal tract, the dominant isoform is γ-SMA (Vandekerckhove and Weber, 1981). As mentioned before, during embryogenesis α-SMA is also transiently expressed in skeletal muscle fibres and in the cardiac myofibrocytes (Woodcock-Mitchell et al., 1988). In addition to smooth muscle, α-SMA is also found in myofibroblasts, myoepithelial cells and pericytes (DeNofrio et al., 1989; Schmitt-Gräff et al., 1994; Willems et al., 1994; Hinz et al., 2001; Chaponnier and Gabbiani, 2004). In contrast to smooth muscle, however, neither of these cells contain h-caldesmon. In myofibroblasts, the amount of α-SMA is the determining factor for the strength of contraction produced by these cells.

The significance of α-SMA expression as one of the contractile isoforms of actin in cells without pathological changes, or in cells of cartilage that have been damaged by various means, remains uncertain. Its role may be in the transference of intracellular tension to the extracellular matrix, which may, among others, influence the tissue-specific arrangement of this matrix (Kim and Spector, 2000; Kinner and Spector, 2002). Concerning the elastic cartilage of the human auricle, chondrocytes expressing α-SMA may cooperate with elastic fibres in conferring the uncommon elasticity of this cartilage and its ability to change shape under the influence of various mechanical forces without damage to its tissue (Kaňa et al., 2006). Our histological findings of the so-called 'layered arrangement' of certain areas of the auricular cartilage concomitantly indicate that the actin-positive chondrocytes may possibly, to a certain degree, assist together with the stratification of elastic fibres to maintain the fixed shape of the pinna.

The described phenomenon of 'layered arrangement' most certainly influences or even directly determines the mechanical attributes of the auricular cartilage. One may for example presume that the differences in the structure of external layers of auricular cartilage may be beneficial during the formation and maintenance of its topography in the areas of concavities and curvatures. In both peripheral layers, the auricular cartilage from flat areas had the same width of actin-positive fusiform chondrocytes. In contrast, the peripheral layers differed in the areas of curvature. On the external, or convex layer of the auricular cartilage, there was a predominance of actin-positive fusiform cells, whereas in the peripheral layer on the concave face, more rounded or polygonal cells were found, which also displayed a positive reaction with α-SMA. In addition, this configura-

tion correlated with the amount of elastic fibres present, as mentioned before in the discussion.

It remains unexplained, however, how two disparate biological materials assembled into one layer will behave, as is the case with the 'layered arrangement' in auricular cartilage. Our analysis of histological tissue blocks of auricular cartilage appears to have brought forth certain detailed findings that lead us to believe that the external configuration of the ear pinna could be directly related to the layered structure of the auricular cartilage. Individual layers are structurally disparate regarding the inclusion of elastic fibres, but also through the differing properties of cellular components of the rearranged chondrocytes of various shapes. This most likely also confers their differing qualities. Despite that, we assume that the so-called 'layering' of the auricular cartilage may play a role in maintaining the original shape. This should be specifically considered during certain plastic surgeries. Undoubtedly, other structures share in the task of holding the known relief of the ear, such as the fatty connective tissue attached to the cartilage, as well as a small number of skeletal muscle fibres.

In our opinion, it is not possible to exclude that the ear cartilage may embody an example of the so-called intelligent biological material, which has its internal structure made in a such way as to more easily develop and yet still maintain all the characteristics of the curvatures of the external ear.

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