Abstract

Introduction

Autologous transplants of the cartilage tissue from the pinna is commonly used in reconstructive surgery of the nasal skeleton. The present study used animal models to elucidate responses of the auricular cartilage to its damage or transplantation to ectopic sites. Histomorphological analysis of changes observed in auricular cartilage including immunohistochemical study of different isoforms of actin and S-100 proteins was performed. Human articular cartilage prepared by in vitro cultivation using artificial scaffolds was also studied after its transplantation.

Aims of the study

The aim was to study histological changes and expression of chondrocytic markers (α-SMA and S-100 proteins) in intact, artificially traumatised, or in a human auricular cartilage cultivated in culture medium. An attempt to grow human auricular cartilage chondrocytes implanted in vitro into various types of three dimensional scaffolds aimed at testing chondrocyte survival and phenotype both in the culture and after transplantation to immunodeficient mice. A human auricular cartilage transplanted into the nasal skeleton of patients during a reconstruction surgery should be submitted to a histomorphological examination. Research assumed also comparison of the auricular cartilage responses to a damage, transplantation or in vitro cultivation, to those of normal, arthrotic, and posttraumatically changed articular cartilage, as well as autologous cultures of articular chondrocytes on scaffolds of ester hyaluronic acids. The study focused at the expression of individual isoforms of actin, S-100 proteins, desmin and some other immunohistochemical markers, in an attempt to contribute to better characterization of differences and similarities between the auricular and articular cartilage responses both to in vitro cultivation and transplantation to ectopic sites.

Material and metods

A total of 162 cartilage samples were studied. This included: 36 samples of a normal cartilage; 5 samples of a pathologically changed human auricular cartilage; 2 samples of a human auricular cartilage transplanted into the nasal skeleton and later removed during a correction surgery; 30 samples of a human auricular cartilage transplanted subcutaneously to immunodeficient mice (NOD129S7 (B6) Rag1) and collected after 12 to 16- weeks (6 intact samples, 18 traumatised samples, and 6 samples cultivated in the culture medium); in addition, 9 samples of cartilage developed in imunodeficient mice transplanted with artificial scaffolds implanted with human auricular chondrocytes; 4 samples of a rabbit auricular cartilage from ear cartilage artificially traumatised; 8 samples of autologous grafts (4 intact and 4 without perichonrdrium) of a rabbit cartilage autotransplanted subcutaneously and collected 8 weeks later'.

In addition we examined 68 samples of a human articular cartilage (56 samples of normal or pathologically changed cartilage and 12 samples of newly formed cartilage after autologous transplantation of chondrocytes (ATC) into articular defects). Histological examinations included immunohistochemical methods using antibodies against α-SMA (α-smooth muscle actin), muscle-specific actin, desmin, and various isoforms of S-100 proteins. In selected cases, RT-PCR was used to examine expression of actin isoforms at mRNA level.

Results

Our research provided, for the first time, a detailed histomorphological description of a layered arrangement of the *auricular* cartilage. The central- and peripheral layers of the auricular cartilage differ in presence and quantity of elastic fibres, as well as in the number, shape and space arrangement of chondrocytes. In addition to S-100 protein, a majority of auricular chondrocytes express α-SMA, particularily in the superficial layer.

Immunohistochemical findings were completed and confirmed by demonstration of the presence of mRNA for α-SMA by RT-PCR. A novel finding was demonstration of rare chondrocytes expressing CD 34, a marker of some adult stem cells.

Expression of S-100 proteins and α-SMA persisted in vital areas of pathologically changed auricular cartilage; in artificially traumatised cartilage; in intact or in vitro cultivated human auricular cartilage transplanted into immunodeficient mice. S-100 proteins and α-SMA were also found in an auricular cartilage which had been autologously transplanted during a reconstruction surgery to replace a missing cartilage in the human nasal skeleton. Human auricular chondrocytes were succesfully cultured using various types of an artificial scaffolding. Subsequently, the chondrocyte implants were transplanted into immunodeficient mice to acces viability of the cells and their potential to generate a cartilage-like tissue. In most cases the procedure resulted in a nodular nest of an elastic type cartilage with chondrocytes that were S-100 protein and α-SMA positive.

In a normal *articular* cartilage α-SMA positive chrondrocytes were present only scarcely in the superficial layers. In contrast to *auricular* cartilage, they became relatively abundant in cases subjected to a mechanical (surgical) damage. A majority of the articular chondrocytes expressed S-100 protein. A tissue culture of human articular chondrocytes grafted onto scaffolding resulted in presence of immature fusiform cells, which had neither histological nor immunohistochemical signs of chondrocytes (e.g.they did not express S-100). However, they were positive for α-SMA. Ten months following the autologous transplantation of aforementioned cultures of chondrocytes on threedimensional scaffolds, a foremostly hyaline type of cartilaginous tissue was formed with areas of a fibrocartilage. Almost all cells, the chondrocytes, were positive for S-100 and α-SMA.

Conclusion

In contrast to the hyaline articular cartilage of joints, a majority of chondrocytes in the auricular cartilage express α-SMA, although their number does not increase after a trauma. Functional significance of α-SMA presence in chondrocytes of normal and damaged cartilage remains unclear. The fusiform arrangement of chondrocytes expressing α-SMA in the elastic cartilage of the ear pinna, together with elastic fibres, may confer and underly the unusual elasticity of the pinna as well as to its ability of undergo significant changes in its shape without being damaged by acting mechanical forces. In contrast to successful transplantation of in vitro cultured autologous articular chondrocytes grown in three dimensional scaffolds and transplanted to sites of joint defects in humans, artificially prepared cartilage which could be used in reconstruction of cartilage defects in the head and neck area is still not available and is a subject of experimental studies, of which the present study is a part. Consequently, in clinical practice method of choice is still transplantation of an autologous cartilage, mostly the cartilage auricular.

Key words: transplantation – implantation – auricular elastic cartilage – articular hyaline cartilage – autologous culture of chondrocytes- scaffold - alpha-smooth muscle actin – S-100 protein