ABSTRACT

Introduction: The role of apoptosis in congenital cholesteatoma (CC) is not completely established. The aim of the study was to analyze the expression of apoptosis-related proteins: p21 and p53 in CC cells.

Material and methods: The immunohistochemistry based on binding of biotinylated secondary antibody with the enzyme-labeled streptavidin with using appropriate primary antibodies in the following tissues: 13 samples of CC, 12 specimens of acquired cholesteatoma (AC) and 12 auditory meatal skin specimens (MS) was performed.

Results: All CC tissues showed a large increase in number of p21-positive cells compared to MS (p<0.05). There was no significant difference in 21-positive cells in CC compared with AC. Considerably difference was found between CC and AC with respect to p53 expression (p<0.05). The distribution of p21 and p53-positive cells in CC epithelium was different from the epidermis. There was no positive staining observed in CC perimatrix.

Conclusions: Up-regulation of p21 protein may play a significant role in CC development and may affect p53-dependent apoptosis. Some differences in molecular pathways of apoptosis between congenital and acquired cholesteatoma are suggested.

INTRODUCTION

Congenital cholesteatoma is characterized by invasive growth and osteolytic activity. The incidence of congenital cholesteatoma is estimated to be between 1% to 5% of all middle ear cholesteatomas. The classic presentation of this lesion is a pearl-like epidermoid cyst primarily localized behind the anterior-superior quadrant of an intact tympanic membrane. There are many theories concerning pathogenesis of congenital cholesteatoma but it still remains controversial and all ideas on its pathogenesis requires more research [3, 4]. According to the one of theories explaining the congenital cholesteatoma develops as a result of proliferation of persistence of not involuted fetal tissue [3]. Analysis of biological activity of congenital cholesteatoma cells may contribute to better understanding of pathogenic pathways in this disorder. The role of molecular and cellular apoptosis traits in congenital cholesteatoma has not been completely established.

RESULTS

P21 protein

Compared to the ear canal epithelium, the percentage of p21-positive cells in congenital cholesteatoma was significantly higher (p=0.0001). In acquired cholesteatoma comparing with control group p21 expression was also significantly higher (p=0.049). There was no significant difference between congenital and acquired cholesteatomas (p=0.81). The proportion of p21-positive cells in congenital cholesteatoma varied considerably, ranging from 22.7% to 80% in different regions of the same cholesteatoma epithelium. The p21-positive cells were distributed in suprabasal layers of cholesteatoma epithelium. In some specimens of congenital and acquired cholesteatoma single staining for p21 was found in basal layer of epithelium. The p21-positive cells were present in ear canal skin mainly in one lower suprabasal layers.

P53 Protein

There was no significant difference between cholesteatomas and control group with respect to p53 expression (congenital cholesteatoma: p=0.96; acquired cholesteatoma: p=0.30). The percentage of p53-positive cells was significantly increased in acquired cholesteatoma compared to that in congenital one (p=0.02). The p53-positive cells were located most commonly in the lower layers of congenital cholesteatoma matrix. In few samples of acquired cholesteatoma, p53 was expressed also in higher suprabasal layers. The skin epithelium samples showed scattered p53-positive cells, mostly in basal and lower suprabasal layers of the epithelium. In the connective subepithelial tissue (perimatrix) no antigen-positive cells were observed in any negative controls. There was significant positive correlation between p53 and p21 expression in congenital cholesteatoma.

METHODS AND MATERIALS

Congenital cholesteatoma samples (n=13) and normal auditory meatal skin (n=12) obtained from patients who underwent surgery for cholesteatoma were included in the study. Acquired cholesteatoma samples were used as a comparable group (n=12). Tissue sections were investigated with the immunohistochemistry technique based on binding of biotinylated secondary antibody with the enzyme-labeled streptavidin with using appropriate primary antibodies. Cells with immunexpression of analyzed antigens: p53 and p21 were defined as antigen-positive. In each section, cells were counted and the percentage of positive cells was determined. The level of significance was set at p<0.05.

CONCLUSIONS

Overexpression of p21 may play a role in CC development and be responsible for accumulation of keratin debris within the ear in the result of cell cycle arrest or apoptosis rate increase. The distribution of cells expressing p21 and p53 congenital is different from normal skin, that suggests deviated biological activity of keratinocytes and show disturbances in apoptosis in this lesion.

DISCUSSION

Several molecular data and DNA analysis show distinction between congenital and acquired cholesteatomas, however, this molecular difference is still unknown and controversial. Excessive process of apoptosis may result in keratin debris accumulation and cholesteatoma masses enlargement [4, 6]. However, this process is also regarded as appropriate answer on cell hyperproliferation and is inseparably associated with keratinization in cholesteatoma epithelium. The p21 can both induce apoptosis, depending on its subcellular locations and other condition. It was proved that p21 expression is depended on wild-type form p53 [1]. Our results indicate that there is positive correlation between p53 and p21 expression in congenital cholesteatoma. It was consonant to the study of Huisman et al., who demonstrated a significant positive correlation between p53 and p21 in acquired cholesteatoma, we observed this correlation in congenital type [2]. In presented study p21-positive cells were observed in suprabasal layers of cholesteatoma epithelium. In healthy skin only one lower suprabasal layer was marked with this marker. These data support the notion that p21 in congenital cholesteatoma may act both as a positive factor in cell growth and the pro-apoptotic stimulator, that influences keratin debris accumulation. We may surmise the important role of p21 in regulating apoptosis in congenital and acquired cholesteatoma epithelium. P53 protein in congenital cholesteatoma may play dual function - it regulates either apoptosis or cell cycle arrest [2]. Huisman et al. showed that there is no increase in apoptosis rate in acquired cholesteatoma, what was determined by active caspase 3 expression and TUNEL method [2]. So it is still plausible that increased cell proliferation is compensated by apoptosis or cell cycle arrest. Kojima et al. demonstrated an increase in proliferation and apoptotic cell death in cholesteatoma. No apoptotic cells were observed in basal cell layer, but retain the capability to undergo apoptotic cell death [3].

REFERENCES


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