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Identification of Potential Biomarkers in Acquired Cholesteatoma

Juliana Gusmão de Araujo[1,2*], Marcela Silvestre Outtes Wanderley[3], Roberto Afonso da Silva[3], Mariana de Carvalho Leal Gouveia[1] and Silvio da Silva Caldas Neto[1]

[1]Department of Otorhinolaryngology, Federal University of Pernambuco, Av. Professor Moraes Rego, 50670-901, Recife-PE, Brazil.
[2]University of Brasilia, University Campus Darcy Ribeiro, 70910-900.Brasília-DF, Brazil.
[3]Laboratory of Immunophatology, Keiso Asami, 50670-90, 1 Recife-PE, Brazil.

Authors' contributions

This work was carried out in collaboration between all authors. Authors JGA, MSOW, MCLG and SSCN designed the study. Authors JGA and SSCN wrote the protocol and the first draft of the manuscript. Authors JGA, MSOW and RAS managed the analyses of the study. Authors JGA and MCLG managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The acquired cholesteatoma, even with all the knowledge accumulated since its first description, still remains a public health problem, far from being solved. A deeper understanding of its pathogenesis is extremely important since it is a destructive lesion that might cause potentially serious complications. We had the objective, in this study, to identify acquired cholesteatoma biomarkers using proteomics platform.
Study Design: descriptive cross-sectional study.
Methodology: Samples were collected from cholesteatoma and also from the retroauricular skin of twelve patients undergoing surgery for cholesteatoma removal. The samples were studied by proteomic analysis, using the Mascot algorithm and the NCBI and Swiss Prot proteins database.

**Corresponding author: Email: gusmao.juliana@yahoo.com;*

Results: Of the 393 spots identified in the analysis of protein extracts of acquired cholesteatoma, only 10 were within acceptable statistical parameters by Mascot algorithm. The proteins detected in acquired cholesteatoma were fibrinogen beta chain, extracellular matrix protein 2, actin cytoplasmic 1, heparan sulfate glucosamine 3-O-sulfotransferase 3A1, tumor necrosis factor alpha 8 induced protein-like 1, stanniocalcin-2, eosinophil lysophospholipase and OFUT1.
Conclusion: Proteins involved in cell migration, regulation of apoptosis, signaling pathways, cellular proliferation, wound healing and inflammatory processes were identified. We were able to draw a proteomic profile of acquired cholesteatoma.

Keywords: Cholesteatoma; proteomics; β-actin; stanniocalcin-2; eosinophil lysophospholipase; extracellular matrix protein 2.

1. INTRODUCTION

Cholesteatoma consists in the presence of keratinized stratified squamous epithelium in any local where this one should not be found on the temporal bone, like middle ear and mastoid cells, locals coated by mucosa [1]. The discussion about how these cells reach the middle ear, still is under discussion. Although many studies have already been conducted, its exact pathogenesis is not fully understood. Theories had already been proposed in order to elucidate the problem, but the fact is that, increasingly, is believed that the appearing and evolution of cholesteatoma are multifactorial events, being related primarily to genetic and molecular characteristics.

According to Caldas and Caldas Neto, in 1988, the expansive and destructive capacity of cholesteatomas may compromise the ossicular chain of the middle ear and eventually cause erosions to the bony protection of the inner ear, facial nerve, meninges and sigmoid sinus, causing irreversible lesions and remote infections that can cause permanent functional disabilities or even death to the patient [2].

Various inflammatory mediators and other proteins had already been related to its etiopathogenesis. Genetic features certainly influenced by some environmental factors, would favor the appearing of cholesteatomas, with its particularities like epithelial migration, invasion of adjacent structures, bone destruction, cellular hyperproliferation, aggressiveness and propensity to relapse.

Thus, studies dedicated to the clarification of this morbidity are extremely important for both scientific interest and for the benefit of public health. Countless researches are in progress and many novelties appear daily about the structure and behavior of cholesteatoma. The discover of the set of molecules that characterize this disease is important to try to determine the probable behavior of the lesion and, so, intervene in its treatment in order to give the patient a greater prospect of cure and decrease the rate of relapse.

Most of the studies that identify proteins related to cholesteatoma are made by means of immunohistochemistry. This method requires the research of one or more predetermined proteins in the studied tissue. However, in this way, several other proteins present in the material may remain unknown until is done a particular research for them. In this context, the proteomics appears as a promising tool in the identification of potential biomarkers of cholesteatoma, because it is a new method that allows the identification of all existing proteins in a given tissue and that may reveal unknown patterns of disease, being potentially useful for early detection and analysis of the evolutionary behavior and assessment of prognosis.

Therefore, the current study has the aim of identify the proteins present in cholesteatoma acquired by means of the use of proteomics platform so in the future, they can be used in studies towards relate them to the clinical features of this disease and the development of new therapeutic methods.

2. METHODOLOGY

Patients with clinical diagnosis of cholesteatomatous chronic otitis media with indication of tympanomastoidectomy by closed or open technique, regardless of age, gender or ethnicity were included. Patients with craniofacial abnormalities, congenital cholesteatoma and cholesteatoma of the external auditory canal were excluded. Samples of cholesteatoma of the middle ear and mastoid and also retroauricular skin fragment of 12 patients undergoing surgery for removal of cholesteatoma in the year 2012

were collected. The samples were stored in saline solution and maintained at -20℃ up to tissue weighing and extraction of proteins.

The proteomic analysis was performed in the Laboratory of Immunophatology Keiso Asami (LIKA) situated in the Federal University of Pernambuco.

2.1 Extraction and Dosage of Total Proteins

The extraction of proteins consisted in the sample incubation in a mix 1:1 of sample buffer for two-dimensional electrophoresis (Urea-5M, thiourea-7M, CHAPS-2%) and cell lysis buffer (CHAPS-1%, Tris-40mM, 10µl/ml of protease inhibitor) followed by 5 cycles of sonication (5 minutes) and ice bath (5 minutes). Then, for the isolation of the supernatant, the samples were subjected to centrifugation (5000 rpm, 10 minutes, 4℃).

Total proteins of the supernatant were precipitated using a 2-D Clean-Up (GE Healthcare Corp., USA) commercial kit, following the manufacturer instructions. The dosage of total protein was performed by the method of Bradford (1976).

2.2 Two-dimensional Electrophoresis

The tapes Immobiline Dry Strips (13 cm, pH3-10) were incubated (16 hours, 25℃) with rehydration solution (urea-7M; thiourea-2M; CHAPS-4%, dithiothreitol-100mM; of bromophenol blue-0.002% and of Pharmalite-2% pH3-10) containning 300 µg of total protein. The isoelectric focalization of the samples dunked in the tapes occurred in the set Multiphor II System da Amersham (GE Healthcare Corp., Piscataway, USA). The programming of first dimension consisted in three steps: 1) 300 V/ 0.001 KVh; 2) 3500 V/ 2.9 KVh; 3) 3500 V/ 12000 KVh. After the 1st dimension, the tapes were balanced with a buffer containning 6M of urea, 50mM of Tris-HCl pH 6.8 buffer, 30% of glycerol, 2% of SDS and dithiothreitol (DTT) during 15 minutes and then using the same buffer and time of incubation, with iodoacetamide (25 mg/ml) replacing DTT. The 2nd dimension was performed by running on 12.5% polyacrylamide gel (SDS-PAGE). The gels were stained with silver nitrate using *Silver Staining Protein* (GE Healthcare Corp., USA) commercial kit, and their images scanned with the aid of a fotodocumentador (Molecular Imaging, Loccus biotecnologia) and analyzed using the software Image Master 2D Platinum 6.0 (GE Healthcare Corp., USA). All analysis were performed in triplicates. The three gels obtained both from the samples of cholesteatoma and the skin were superposed to determine its reproducibility.

2.3 Digestion of Spots

First, the spots of interest were cut with the aid of a sterile scalpel and added to tubes containing 1 ml of destaining solution (potassium ferricyanide-30 mM and sodium thiosulfate 100 mM). After 5 minutes, it was performed the washing with ultrapure water until the disappearance of the yellowish color. Then, by the addition of acetonitrile 100%, began the dewatering of the spots. The acetonitrile was removed by *Speed Vac* (Concentraton 5301, Eppendorf). The trypsin was prepared according to the instructions of the manufacturer (Invitrogen Inc, USA) at 20 µg/ml in a buffer NH4HCO3 25mM. For activation of the enzyme, the spots were incubated at 37℃ during 16 hours. After the end of digestion the enzyme was inactivated and the organic extraction of peptides by the addition of 30 µL of 5% TFA: 50% ACN solution was started. After 1 hour, the solution containing the extracted peptides was transferred to a new tube and concentrated with the aid of *Speed Vac.*

2.4 Mass Spectrometry

In this study, Bruker Autoflex III MALDI- TOF (Bruker Corporation) mass spectrum was used, equipped with solid phase laser, Nd:YAG (355 nm), quadratic field refletron and timed ion gateway. The acquisition of the intact peptides was performed on *reflection* mode, with positive ionization, mas rejection 500 m/z, acquisition speed of 200 shot/sec, accelerating potential of 20 kV ions, being collected 1000 shots for each spectrum. After extraction of the peptides, the samples were diluted in 10 µL of the solution at 1% of trifluoroacetic acid (TFA) and 50% of acetonitrile. After this procedure an aliquot of de 2 µL was mixed with the same volume of the matrix acid 4-a-hydroxide cinnamic (5 mg/ml) and 1 µL was applied on the slide with drying at room temperature. The slide was inserted into the mass spectrometer to obtain spectra. The external calibration was performed with standard calibration kit (Bruker Corporation), using the following patterns:

- Angiotensin II (M+H 1046, 5418 monoisotopic),

- Angiotensin I (M+H 1296, 6848 monoisotopic),
- P substance (M+H 1347, 7354 monoisotopic),
- Bombesin (M+H 1619, 8223 monoisotopic),
- ACTH clip 1-17 (M+H 2093, 0862 monoisotopic),
- ACTH clip 18-39 (M+H 2465, 1983 monoisotopic) and
- Somatostatin 28 (M+H 3147, 4710 monoisotopic).

The graphs were plotted in Flex Analyses software (Bruker Corporation). All analysis were performed in triplicates. The obtained spectra were analyzed using the algorithm Mascot (Matrix Biosciences) over the protein database of NCBI and Swiss Prot. The parameters used were: Taxonomy: *Homo sapiens,* fixed changes: carbamidomethyl (C), variable changes: Oxidation (M).

After the identification of proteins that characterized cholesteatomatous tissue, we made a detailed description of them in order to establish a proteomic profile of the disease.

3. RESULTS

The age of the patients who had samples collected ranged from 09 to 70 years, been the average 29.92 years old. There were seven women and six men.

The samples of cholesteatoma and skin weighed between 40-100 mg and the hole piece was submitted to extraction of total proteins. The samples of cholesteatoma presented on average 5000 µg/ml and the ones of skin 3000 µg/ml. And aliquots of 300 µg were sent to two-dimensional electrophoresis

In this study, the adopted tool of proteomics, electrophoresis 2-D, identified about 500 spots, being 393 in the analysis of acquired cholesteatoma protein extract and 108 in the sample of skin (Figs 1 and 2). The 126 spots considered more intense, of which 80 were present in the samples acquired cholesteatoma, were subjected to enzymatic digestion for extraction of peptides. These peptides were taken to mass spectrometry to obtain the spectra, and of the 80 spots initially obtained, only 10 were within the statistical parameters acceptable by this process (Table 1). The proteins identified after analysis of the spots present in the skin were used as control in the research for the establishment of which molecules exist only in cholesteatoma and not in normal skin, seeking in this way to draw a more reliable proteomic profile of the pathological process.

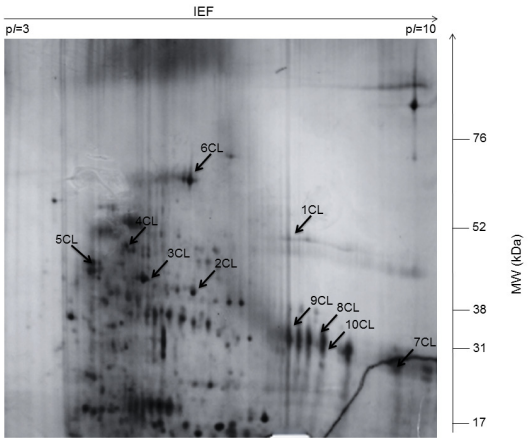

Fig. 1. Analytical two-dimensional electrophoresis gel from protein extract of acquired cholesteatoma stained with silver nitrate. The spots were numbered according to Table 1

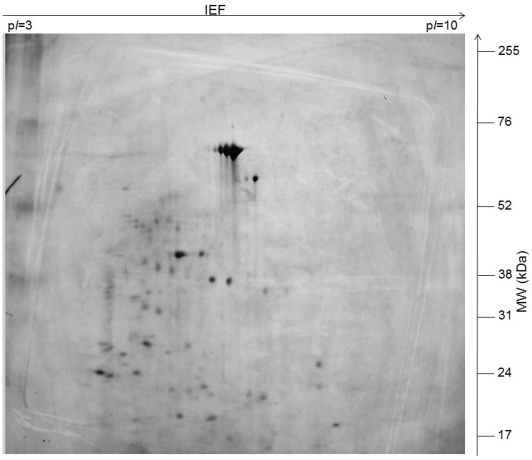

Fig. 2. Analytical two-dimensional electrophoresis gel from protein extract of acquired cholesteatoma stained with silver nitrate

Table 1. Proteins identified in cholesteatoma by MALDI-TOF from enzymatic digestion of spots of two-dimensional electrophoresis

Spot	Protein	Coverage	Scores	Matches	p*I*	MW (Da)
1CL	Fibrinogen beta chain	63%	95	18	7.57	47543
2CL	Extracellular matrix protein II	20%	66	15	5.36	37020
3CL	Actin, cytoplasmic 1	49%	68	15	4.47	38256
4CL	Keratin 14	50%	104	37	4.13	43176
5CL	Heparan sulfato glicosamina 3-O-sulfotransferase 3A1	24%	58	12	3.70	36264
6CL	Putative ankirin repeat domain-containing 26-like protein	19%	65	12	5.13	59501
7CL	Tumor necrosis factor α induced protein 8-like 1 (TIPE1)	18%	62	9	9.26	31343
8CL	Stanniocalcin-2	21%	62	13	7.55	31516
9CL	Eosinophil lysophospholipase	33%	58	48	7.34	32799
10CL	GDP-fucose protein O-fucosyltransferase 1	30%	62	52	7.84	31730

The protein scores greater than 56 are significant (p<0.05)

As a result, from 10 proteins identified in the samples oscholesteatoma, one, keratin 14, was excluded due to its presence in the retroauricular skin. Therefore, by also exist in normal skin, we do not consider it in the study.

The analysis by mass spectrometry identified in cholesteatoma, proteins involved with cell migration (β-actin, heparan sulfate glucosamine 3-O- sulfotransferase 3A1 and fibrinogen beta chain), control and regulation of apoptosis (tumor necrosis factor α-induced protein 8-like 1), inflammatory processes (eosinophil lysophospholipase and fibrinogen beta chain), cellular signaling pathways (GDP-fucose O-fucosyltransferase 1), healing and tissue repair (Heparan sulfate, stanniocalcin), and tissue hyperproliferation (stanniocalcin). Besides these functions, most of them are indirectly involved in carcinogenic processes acting sometimes as tumor markers. It was also identified the extracellular matrix protein 2, important component of the extracellular matrix. We also found the "putative ankirina repeat domain-containing protein 26-like protein".

4. DISCUSSION

Many studies have been conducted about the cholesteatoma, in an attempt to discover new aspects of the pathogenesis of the disease. In this context, researches dedicated to clarifying the molecular biology involved in cholesteatoma are valuable for understanding its cause, variability of presentation and aggressiveness. Among these studies, many works using immunohistochemical methods made possible the more detailed analysis of some proteins and their participation in the cholesteatoma etiopathogeny.

However, researches using immunohistochemistry are limited by the small number of identified proteins. It is due to the non global nature of this approach, since this technique is capable to identify only proteins defined in advance. Thus, the scientific community is intensifying efforts for the padronization of wide techniques, using simpler biological samples and with low cost.

Proteomics seeks to relate the tissue activity to the set of proteins expressed at a given moment, since it is able to directly identify proteins. In this way, it becomes possible identify new molecules that indicate changes from normal physiological state to the pathological, capable to act as markers in the disease process. Without this technique, we would be limited to proteins already thoroughly investigated and with available and affordable technology to immunohistochemistry studies.

Not taking into consideration its pathogenic basis, all types of cholesteatomas share the same properties. Their common clinical characteristics are invasion, migration, hyperproliferation, change in cellular differentiation, aggressiveness and tendency to recurrence.

In this study, it was evaluated the proteomic profile of cholesteatoma. Using techniques that have followed well stablished methodologies and are routinely performed on LIKA, 10 proteins

were identified. From these proteins, keratin 14 was excluded due to its presence in the retroauricular skin used as control in the research. Therefore, as it was also identified in healthy skin, we do not consider it in the study. Besides this, it was identified the *putative ankirina repeat domain-containing protein 26-like protein*, whose existence is questionable and it was also excluded from the study.

Among the proteins found only in cholesteatoma, we initially highlight the cytoplasmic actin 1, also referred to as β-actin (3CL). Actin is an essencial component of the cytoskeleton, performing a critical role in a wide variety of cellular processess, including cell migration, cellular division and regulation of gene expression. These functions are attributed to the ability of actin to form filaments, that can fastly set up and pull apart according to the needs of the cell [3]. B-actin is its main isoform and is responsible for drive cell migration, being crucial on embryogenesis, on healing and immune response [4]. The increased expression of β-actin estimulates the cell migration and its concentration is greater on migrating cells margins [5].

Among the theories proposed for the acquired cholesteatoma of the middle ear, one of the most important considers the epithelial migration as the source of the disease. This migration would begin from the margins of a tympanic membrane perforation, or from its retractions [6]. The presence of β-actin in the cholesteatoma samples gives support to this theory.

Cholesteatoma presents cells of the base layer of the epithelium with capacity to form pseudopods and through them is promoted to cell migration and growing of cholesteatoma [7]. We suggest that the β-actin found with higher intensity in the acquired cholesteatoma samples is compatible with the epithelial migration, once its polymerisation guides this migration. The presence of β-actin in the cholesteatoma samples would justify the greater predisposition of epithelial tissue in these patients to migrate toward the middle ear causing accumulation of skin scales in this region.

The protein heparan sulfate glucosamine 3-O-sulfotransferase 3A1 or 3-OST- 3A (5CL) was also found in our samples. It is an enzyme responsible for the synthesis of heparan sulfate, a glycosaminoglycan [8].

The heparan sulfate, found in various tissues is responsible for a variety of physiological responses, such as development and cell growth, cell migration and wound healing [9]. This molecule regulates a variety of biological activities, including angiogenesis, blood clotting and the emergence of tumor metastases.

Furthermore, it was also demonstrated that, the heparan sulfate glucosamine 3-O-sulfotransferase 3A1, during the inflammatory processes, interacts with the heparin binding proteins, performing a key role in leukocyte extravasation and chemotaxis. It also carries out the function to promote the release, by the macrophages, of cytokines, as TNFα, IL-1 and IL-6 [10].

As had been previously described, the cholesteatoma cells demonstrated an increase in IL-1, IL-6 and TNFα, cytokines that have biological activities in cell proliferation and bone metabolism [11,12]. Other authors have also reiterated the production of these cytokines by cells of cholesteatoma [13,14]. IL-1 and IL-6 estimulate bone resorption, increasing the number of osteoclasts precursor cells [15]. The high concentration of osteoclasts was associated to the erosion of the ossicular chain and the presence of intraoperative granulation tissue [12]. TNFα acts could also act, in the cholesteatoma, as a mediator in the process of destruction and remodeling of the tissue, increases the activity of collagenase, exposing the bone surface to action of osteoclasts. The concentration of TNFα is proportional to the level of bone destruction [16]. It also estimulates the protein synthesis, proliferation and differentiation in the production of keratin [17].

This way, the presence of heparan sulfate glucosamine 3-O-sulfotransferase 3A1 in the cholesteatoma samples is coherent with the works previously published. Its action in increasing the release and action of these cytokines involved in the pathophysiology of cholesteatoma puts it as a potential biomarker for this disease.

Following the investigation of inflammatory mediators, we found increased expression in the cholesteatoma of the tumor necrosis factor α induced protein 8-like 1 or TIPE1 (7CL), that seems regulate cell death, having a significant role in immune homeostasis and development of cancer [18].

Jian et al, in 2011, examined the expression of TIPE1 in tissues of rodent and human cells using immunohistochemistry, polymerase chain reaction and *Western blot*. High levels of TIPE1 were detected in carcinoma cell lines, specially in cells transformed by viral genomes [19]. These results suggest that TIPE1 can execute functions of carcinogenesis, mainly due to its activity in regulating cell death.

In cholesteatoma, apoptosis is markedly enhanced when compared with healthy skin. The hyperproliferation of epithelial cells of cholesteatoma is counteracted by the increase in the rate of programmed cell death, which differs from squamous cell carcinoma, in which the increased cell proliferation, without a compensatory increase of apoptosis may be associated with malignant transformation [20]. These data make us believe that the presence of TIPE1 in cholesteatoma samples may work as a cell death marker, allowing us to infer, in the future, the level of cell hyperproliferation and aggressiveness of this disease.

Another protein identified was the fibrinogen beta chain (1CL). Fibrinogen is a glycoprotein formed by three pairs of non-identical polypeptide chains: chains *alpha*, *beta* and *gamma* [21]. After vessel injury, fibrinogen is cleaved by thrombin to form fibrin. Furthermore, many cleaved products of fibrinogen and fibrin regulate the cell adhesion and its proliferation, have vasoconstrictor and chemotactic activities, and are mitogens for several cell types [22].

Fibrinogen plays a crucial role in hemostasis, inflammatory reactions, tissue repair, healing and angiogenesis, improving cell adhesion, migration and differentiation of endothelial cells [22]. It is known as a inflammation marker, as well as C-reactive protein and some proinflammatory cytokines. This justifies the appearance of beta chain of fibrinogen in cholesteatoma samples of our study, since the inflammatory process in this disease is evident.

Another protein found in acquired cholesteatoma was eosinophil lysophospholipase or Charcot-Leyden crystals protein (9CL), produced by transcription of CLC gene. This protein corresponds to about 10% of the total cell proteins of eosinophils and basophils. Its identification in body fluids is considered an allergy inflammation marker associated to eosinophils [24]. In the field of otolaryngology, this protein has already been isolated in patients with allergic rhinitis and nasal polyps [28,29], diseases in which is well known the role of eosinophils. It is believed that eosinophil mediators, such as eosinophil lysophospholipase, are responsible for epithelial damage of the sinus mucosa through their cytotoxic effects [25,26]. But, in cholesteatoma, it was found for the first time and this fact enlarges the perspective of new cells and inflammatory mediators researches that may be involved in the etiopathogenesis of this disease.

Another protein found in cholesteatoma was a glycosyltransferase, a GDP-fucose O-fucosyltransferase 1 or OFUT1 (10CL), which carries out a crucial role in the *Notch* signaling pathways through the changes performed in the epidermal growth factor constituents of their receptors. *Notch* proteins are surface receptors for transmembrane links that regulate some cellular mechanisms, carrying out crucial function in the development of many human diseases, including types of cancer [27].

Recent experiments showed a suppressive effect of the *Notch* transmembrane receptors in the differentiation of osteoblasts and osteoclasts, making this signaling pathways to carry out key role in the bone development process and its neoformation [28]. It is believed that, in cholesteatoma, bone defects are resulting from active resorption process [23]. The mechanism of this absorption, however, is not completely understood. The presence of OFUT1 in our samples and its function in the *Notch* signaling pathways reaffirms the important and essential activity of this signaling pathways in the process of erosion and bone remodeling so intensely observed in patients with cholesteatoma.

There is a considerable increase in the number of osteoclast precursor cells in the perimatrix of the cholesteatomatous tissue, and the osteoclasts carries out crucial function of this process of bone resorption in cholesteatoma[29]. The presence of OFUT1 in cholesteatoma samples and its role in the differentiation of osteoclasts and osteoblasts ratifie the participation of these cells in the process of bone resorption in this disease. OFUT1 protein emerges in this context, as a possible biomarker for the destruction of the underlying bone tissue to the acquired cholesteatoma.

Another protein detected in cholesteatoma samples was stanniocalcin 2 (8CL), glycoprotein whose exact function is still unknown [30].

Volland et al. [31], observed that stanniocalcin is associated to a higher activity of matrix metalloproteinase 2 (MMP2). Metalloproteinase are proteolytic enzymes responsible for bone homeostasis showing an increase in its concentrations in osteolytic inflammatory diseases. It was already demonstrated MMP2 expression in cholesteatoma and that individuals with complications had a more intense immunohistochemical expression of MMP2, suggesting the possibility that it may activate mechanisms of bone invasion by cholesteatomas [32,13,33,34]. Stanniocalcin 2 may play an important role in this process, once it may be associated to a higher activity of MMP2. Therefore, stanniocalcin has potential to become an excellent biomarker of cholesteatoma.

It was already demonstrated that osteoblasts, produce significant amount of stanniocalcin 2, [35]. These factors confirm the potential role of stanniocalcin 2 as a molecular marker of cholesteatoma, once its role in bone metabolism, essential in the cholesteatoma pathophysiology, has already been described.

Still in this study, it was observed that the extracellular matrix protein II or matrix glycoprotein SC1/ECM2 (2CL) has also been found in cholesteatoma samples. This protein is an important component of the extracellular matrix, belonging to the family of "small leucine-rich proteoglycan" and its physiological function is strictly related to the aggregation and cellular adhesion [36].

Quantitative and qualitative changes of extracellular matrix components may be induced by cytokines that change cellular metabolism of the connective tissue. Together with immunoglobulins is capable to increase the proliferation of B cells, besides increasing lymphopoiesis when expressed as a transmembrane protein in fibroblasts [37]. The production of these mediators can be deflagrated by the penetration of submucosal space by middle ear infection, as well as by cell necrosis.

The proteins above described, found in cholesteatoma samples have not been previously identified in this disease. Despite this, papers published about their participations in cellular and tissue function, make the characteristics of these proteins fully compatible with the theories already published about the pathophysiology of cholesteatoma (Table 2). These findings, therefore, open space for a more reliable characterization of cholesteatoma molecular parameters and extend the possibilities of new studies that allow us understand more clearly the mechanisms that take to the appearance, evolution and perpetuation of this type of disease.

There is still the expectation that useful biomarkers for the diagnosis and monitoring of cholesteatomatous lesions, through the use of serum enzymatic assays or even biosensors, as in hemoglucoteste for diabetic patients. Researches will continue to be performed in order to allow a better assistance for patients.

Table 2. Summary of proteins identified in cholesteatoma and their functions

Protein	Summarized functions	Potential role in cholesteatoma
β-actin	Cell migration, healing and immune response	Epithelial migration
Heparan sulfate glucosamine 3-O-sulfotransferase 3A1	Cell growth and release of cytokines (TNFα, IL-1 and IL-6)	Cell proliferation and bone metabolism
Fibrinogen beta chain	Hemostasis and inflammatory reactions	Inflammation marker
Tumor necrosis alpha factor induced 8-like 1 protein	Immune homeostasis and cell death regulation	Apoptosis characteristic of hyperproliferative diseases
OFUT1 protein	Differentiation of osteoclasts and osteoblasts	Bone resorption
Stanniocalcin 2	Increases MMP2 activity	Bone resorption
Eosinophil lysophospholipase	Allergic inflamation	Potential role of eosinophil in the cholesteatoma pathogenesis
Extracellular matrix protein 2	Component of the extracellular matrix related to the aggregation and cellular adhesion	Extracellular matrix protein

However a limitation of this study was that the cholesteatoma samples were clumped in to one and the proteomic analysis was not performed for each individual separately.

5. CONCLUSION

The main proteins found in cholesteatoma were β-actin, heparan sulfate glucosamine 3-O-sulfotransferase 3A1, fibrinogen beta chain, tumor necrosis alpha factor induced 8-like 1 protein, OFUT1 protein, stanniocalcin 2, eosinophil lysophospholipase, and extracellular matrix protein 2.

Based on these findings, the proteomic profile of acquired cholesteatoma was made, identifying proteins still not isolated in this pathology and enabling a more reliable characterization of its molecular parameters. In the future, it is expected to be possible, through new researches, correlate the proteins identified with the clinical findings of the disease and the development of new propaedeutic and therapeutic methods.

CONSENT

All authors declare that written informed consent was obtained from the patients for publication of this article.

ETHICAL APPROVAL

The research protocol was approved by the Ethics Committee of the Hospital das Clínicas da Universidade Federal de Pernambuco, with the processes numbers 133346 and 141292. All patients included in the study were informed about the aim and procedures involved in the research and signed a Free and Clarified Consent Term.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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