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SHORT COMMUNICATION

In silico analysis for drug repurposing in cholesteatoma: A novel approach to address an unmet medical need

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Abstract

Cholesteatoma presents a significant clinical challenge with limited pharmaceutical treatment options. This paper aims to explore the potential of drug repurposing for managing cholesteatoma through advanced bioinformatics analysis of high-throughput genetic data. For this purpose, we conducted a systematic search of published high-throughput genetic studies related to cholesteatoma and used functional and literature enrichment analysis of multiple sets, a validated web-based bioinformatics platform, for analysis. We employed common pathway enrichment analysis and cross-referenced data from DrugBank and gene list automatically derived for you drug annotations to identify potential medical treatments. Our analysis covered eight high-throughput genetic studies, with extended gene lists available for five of them. Enrichment analysis identified common pathways, including matrix metalloproteinases, interleukins, apoptosis, and the phosphoinositide 3-kinase-AKT pathway, shedding light on both expected and less-studied aspects of cholesteatoma pathogenesis. In addition, the analysis proposed several medications, including anti-tumor necrosis factor-α (TNF-α), zinc, and marimastat, as potential treatments. In conclusion, drug repurposing is a potential cost-effective approach to address the unmet medical need for cholesteatoma management. The identified medications, especially anti-TNFα and zinc, offer promising options. Given the limited research funding in this field, this bioinformatic approach holds great promise, highlighting specific molecular pathways that hold the greatest potential to be implicated in the pathogenesis of cholesteatoma and offering a faster route for future trials to reduce cholesteatoma recurrence.

Keywords: Cholesteatoma; Enrichment pathways; Drug repurposing; Bioinformatics platform

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1. Introduction

Cholesteatoma, a keratinizing epithelial lesion within the middle ear, poses a significant clinical challenge due to the lack of pharmaceutical treatments. At present, surgical

intervention remains the primary strategy for managing cholesteatoma, but it is far from ideal. Recurrence rates, soaring up to 40%, continue to be a challenging problem and the invasive nature of surgery places the structures of the temporal bone at risk.[1-3] This underscores the pressing need for a more effective, non-invasive medical approach to cholesteatoma management.

The pharmaceutical industry's prioritization of drug development has left cholesteatoma largely overlooked. To date, there are no investigational drugs specifically designed for this condition. However, signs of hope emerge through the field of drug repurposing, also known as drug repositioning. This innovative approach seeks to identify new therapeutic applications for existing drugs, whether approved for other indications or previously failed in clinical trials.[4] By investigating this unexplored opportunity in the field of pharmaceuticals, we may uncover a medical treatment that could revolutionize cholesteatoma management.

Even though the cholesteatoma's pathophysiology remains largely unclear, a surge in genetic research, driven by high-throughput techniques, has yielded a wealth of data. Thousands of gene expression profiles and non-coding microRNAs have been studied.[5] Yet, the underlying mechanisms of cholesteatoma development and progression remain largely unknown.[1,2] At this point, bioinformatics, based on the advances of computer, have the potential to make sense of all this genetic information.

In this regard, this study aims to utilize advanced bioinformatics to carefully examine the extensive genetic data accessible for cholesteatoma. By thoroughly exploring this abundance of information, our goal is to identify the likely molecular pathways responsible for cholesteatoma formation, progression, and recurrence. Armed with these insights, we seek to propose the repurposing of drugs associated with these identified pathways. This interdisciplinary approach merges genetics, bioinformatics, and pharmacology, offering a promising avenue for cholesteatoma treatment innovation.

2. Methods

Published high-throughput genetics studies were searched in the PubMed database. Genes or other genetic factors that can be retrieved by these publications and their corresponding databases were then introduced in functional and literature enrichment analysis of multiple sets (FLAME), a novel validated web bioinformatics platform, which is freely available to public.[6,7] FLAME excels in the simplicity of use, providing extensive flexibility in importing and managing multiple lists, executing advanced state-of-the-art functional enrichment analysis pipelines, and merging and prioritizing results through sophisticated interactive visualizations. In our study, FLAME was utilized to correlate gene lists at a network level, leveraging resources such as aGOtool, g:Profiler, WebGestalt, and EnrichR. Common enrichment pathways between the high-throughput cholesteatoma genetic studies were retrieved by utilizing the aforementioned tools. The number of times a pathway was found, and the higher enrichment scores were extracted. In addition, the option to enrich the provided gene lists from DrugBank and gene list automatically derived for you (GLAD4U) drug annotations were chosen to find potential medical treatment options. The statistical significance of the combined results is estimated by applying Fisher's combined probability test represented by X^2 and combined P-value metrics for each enrichment term identified by two or more tools.

Studies on specific genetic factors and pathways were excluded from the analysis since the aim of this *in silico* analysis is to enrich current knowledge, or in other words, to suggest possible new molecular targets in cholesteatoma research and treatment.[8]

3. Results

Of the nine high-throughput genetic studies that were identified, extended gene lists were available in six.[8-13] In three studies,[14-16] a full list of genetic factors could not be retrieved in the published paper and after contacting the corresponding author.

Overlapping enriched that can be found in at least two studies is shown in Table 1.

Drugs or substances proposed following this *in silico* analysis as potential robust modulators of the enriched metabolic pathways are shown in Table 2.

4. Discussion

In the present study, we focused on utilizing high-throughput studies as they provide a multifaceted set of genetic data. This approach was chosen because studies analyzing only a few genes may introduce selection bias and may not require advanced bioinformatic techniques to reveal potential pathways. By employing high-throughput data, we aimed to reduce the risk of bias and gain a more complete understanding of the genetic factors associated with cholesteatoma.

The limitation of this analysis is that we primarily relied on expression studies. While these studies offer valuable insights, they cannot definitively establish whether the altered gene expression is a primary cause or a secondary effect of cholesteatoma. Consequently, it remains uncertain

Table 1. Enriched pathways found in common in at least two studies

Pathways	$-\chi^2$	log10 (*p*-value)
Pathways common in four studies		
Matrix metalloproteinases and their activation	4.25E−06	5.37
PI3K-Akt signaling pathway*	0.00000446	5.35
Apoptosis	7.12E−06	5.15
Collagen degradation	1.12E−05	4.95
Degradation of extracellular matrix	1.24E−05	4.91
Extracellular matrix organization	7.48E−05	4.13
Vitamin D receptor pathway	0.0017	2.77
Cell surface interactions at vascular wall	0.00629	2.2
Pathways common in three studies		
Innate immune system	1.23E−10	9.91
Metal sequestration by antimicrobial proteins	2.27E−06	5.64
Antimicrobial peptides	0.000133	3.88
Formation of cornified envelope	0.0268	1.57
Pathways common in two studies		
IL-18 signaling pathway	2.2E−08	7.66
Keratinization	5.71E−07	6.24
Metabolism of lipids	6.62E−07	6.18
CCKR signaling map ST	1.09E−06	5.96
Cytokine signaling in immune system	3.22E−06	5.49
Neutrophil degranulation	4.33E−06	5.36
Signaling by interleukins	1.12E−05	4.95
Interleukin-4 and interleukin-13 signaling	1.23E−05	4.91
IL-1 and megakaryocytes in obesity	9.09E−05	4.04
Focal adhesion	0.000217	3.66
Toll-like receptor 4 (TLR4) cascade	0.000559	3.25
Lung fibrosis	0.000873	3.06
Cell junction organization	0.00219	2.66
Signaling by receptor tyrosine kinases	0.00568	2.25
Signaling by nuclear receptors	0.0103	1.99
Type I hemidesmosome assembly	0.0193	1.71
Estrogen signaling pathway	0.0209	1.68
MyD88:MAL (TIRAP) cascade initiated on plasma membrane	0.0248	1.61
Fibronectin matrix formation	0.028	1.55
Collagen formation	0.0374	1.43

*The only pathway found in common in five studies.
Abbreviation: IL: Interleukin.

whether the identified pathways are directly implicated in the disease's pathogenesis or are simply induced by the inflammation associated with cholesteatoma.

Table 2. Potential useful substances for the cholesteatoma management proposed by our primary *in silico* analysis

Study	Source	Function	-log10 (*p*-value)
Lee *et al.*[12]	DRUGBANK	Zinc	5.1
	GLAD4U_DRUG	Collagenase	4.87
	GLAD4U_DRUG	Protease inhibitors	4.55
	GLAD4U_DRUG	Papain	4.53
	GLAD4U_DRUG	Ustekinumab	4.12
	GLAD4U_DRUG	Alteplase	3.87
	GLAD4U_DRUG	Urokinase	3.83
	GLAD4U_DRUG	Interleukin inhibitors	3.8
	DRUGBANK	Calcium	3.67
Klenke *et al.*[13]	GLAD4U_DRUG	Infliximab	4.95
	GLAD4U_DRUG	Heparins or heparinoids for topical use	4.87
	GLAD4U_DRUG	Heparin	4.87
	GLAD4U_DRUG	Nystatin	3.92
	GLAD4U_DRUG	Other hormones	3.89
	DRUGBANK	Zinc	3.85
	GLAD4U_DRUG	Etanercept	3.82
Kwon *et al.*[15]	DRUGBANK	Marimastat	6.7
Tokuriki *et al.*[10]	GLAD4U_DRUG	Interleukin inhibitors	15.65
	GLAD4U_DRUG	Heparins or heparinoids for topical use	5.13
	GLAD4U_DRUG	Collagenase	4.56
	DRUGBANK	Marimastat	3.4
	GLAD4U_DRUG	Other hormones	3.13
	GLAD4U_DRUG	Protease inhibitors	3.43

Note: The study of Yoshikawa *et al.*[8] was the only high-throughput study targeting specific cells (fibroblasts) of cholesteatoma. An extensive list of potential therapeutic agents was retrieved by our analysis. Only common agents with the rest of the studies are reported here.
Abbreviation: GLADU: Gene list automatically derived for you.

Nevertheless, certain findings, such as the involvement of matrix metalloproteinases (MMPs) and interleukin (IL) pathways, which have been previously observed in cholesteatoma research, are not unexpected from this analysis. Several published studies since the middle 1990s indicate that MMPs could play an active role in the molecular mechanisms of cholesteatoma invasion into the temporal bone.[17] Since then, animal models,[18] *in vitro*

cultures[19], and direct expression studies on cholesteatoma tissues[20-23] have been utilized to decipher the complex molecular interplay of proinflammatory pathways in relation to metalloproteins, osteoclasts activation, and bone resorption. Most of the above indicative references conclude that various MMPs are related to disease aggressiveness or recurrence. There are indications of dysregulation of local immune status in cholesteatoma, as suggested by the expression studies of several ILs and other cytokines[24,25] or the study of various signaling pathways such as those involving triggering receptor expressed on myeloid cells-2 and toll-like receptor 4.[18] Nevertheless, a partial understanding of the proinflammatory mediators and MMPs in bone resorption has been achieved, as indicated by, for example, the conflicting results published regarding the promising role of the RANKL/orthopantomogram pathway.[26,27]

In addition, our approach enriched less well-studied pathways of cholesteatoma pathogenesis, such as apoptosis and the PI3K-AKT pathways and provided some less-expected insights, such as the finding of interactions at vascular wall. There is an increasing body of evidence that epithelial keratinocytes in cholesteatoma are protected against apoptosis.[28-30] There are also some indications of the presence of drivers of angiogenesis,[31] although the exact role of these factors in the perimatrix of cholesteatoma is largely unknown. The notable aspect of these studies is that they provide a molecular framework and do not dispute common mechanical theories of cholesteatoma formation, such as the retraction pocket mechanical theory.

Our analysis also led to the identification of medications that either have previously been suggested for cholesteatoma treatment, such as anti-tumor necrosis factor α (TNFα), or are unexpected, such as zinc or heparin. Notably, there is a case report suggesting the potential effectiveness of anti-TNFα in cholesteatoma management.[32] TNFα, a multifunctional proinflammatory cytokine implicated also in lipid metabolism, coagulation, insulin resistance, and endothelial function, seems to be involved in several of the proposed molecular pathways extracted from our analysis.

In addition to these findings, our analysis proposed other substances, such as zinc and marimastat, which hold promise in cholesteatoma treatment. Of note is that the bioinformatic programs utilized here for the discovery of potential medical treatments are well-known and freely available, but they do not utilize artificial intelligence for the elaboration of the expanding knowledge at molecular level. At present, several companies are developing their own proprietary algorithms for drug repurposing and have already provided important results in other diseases. Here, a primary reading of the suggested molecular pathways can lead to the proposal of various other medications such as duvelisib, an anti-PIK3 medication. This inhibitor has been suggested previously,[3] since the PI3K-Akt signaling pathway enriched by our method has also been proposed in cholesteatoma pathogenesis in a separate study focusing only on molecules involved in this pathway.[28]

This information is particularly valuable given the limited research funding allocated to cholesteatoma. The discovery of a dedicated medical treatment for cholesteatoma within the next few years is unlikely. However, drug repurposing offers an efficient and cost-effective pathway toward this goal. Relying on drug repositioning for the development of cholesteatoma management strategies is a pragmatic approach, considering the challenging research landscape.

Furthermore, some of the medications identified in our analysis could potentially be applied topically during initial treatment to assess their impact on reducing cholesteatoma recurrence. For example, incorporating zinc into materials used in cholesteatoma procedures, such as bone-alive or gelfoam, could serve as a practical trial to evaluate its effectiveness in reducing recurrence rates.

Finally, our study highlighted the utility of FLAME, a validated open-source program capable of integrating complex genetic data. While FLAME's application extends to various diseases, it particularly proves invaluable in the context of cholesteatoma, as demonstrated in our research. This tool has the potential to advance our understanding of the genetic underpinnings of the pathological condition and may have broader applications in medical research beyond cholesteatoma.

5. Conclusion

Functional enrichment analyses are particularly promising for underfunded research areas, as they can suggest potential avenues for drug repurposing. Our study highlights the value of high-throughput approaches and the integration of bioinformatic tools in elucidating cholesteatoma pathogenesis, both by confirming known pathways and identifying novel therapeutic targets.

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Conflict of interest

The authors declare no conflict of interest.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Data are available from the corresponding author upon reasonable request.

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