ARQUIVOS DE ASMA, ALERGIA E IMUNOLOGIA

Original Article

The impact of gut microbiota on thyroid immune response: bioinformatics analysis

O impacto da microbiota intestinal na resposta imunológica da tireoide – avaliação com ferramentas de bioinformática

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ABSTRACT

Introduction: Perturbations in the composition and functionality of gut microbiota have been implicated in various autoimmune diseases, including those affecting the thyroid gland, but the exact mechanisms are still unclear, despite recent progress in research. **Objective:** To investigate the influence of gut microbiota on thyroid immune response through bioinformatics analysis. **Methods:** The study used genomic and proteomic sequence data from the National Center for Biotechnology Information database to examine the influence of gut microbiota on thyroid immune response. Taxonomic analysis and metagenomic analysis were performed, and functional annotation tools were used to identify sequences and pathways involved in immune response. Biological networks were constructed to understand the complex biological interactions. **Results:** Sequences of 944 microorganisms specific to thyroid immune response were obtained from the National Center for Biotechnology Information. The taxonomic composition consisted of Bacteroides stercoris (35%), *Agathobacter rectalis* (25%), *Prevotella jejuni* (15%), and *Roseburia* (10%), and smaller percentages of *Bifidobacterium faecale*, *Clostridium coccoides*, *Clostridium leptum*, and *Lactobacillus iners*. The taxonomic diversity index was high (0.85). Biological networks were constructed, revealing 8 significant modules. The NF-kB and MAPK signaling pathway was identified as specifically influencing gut microbiota, playing a critical role in modulating

RESUMO

Introdução: Perturbações na composição e funcionalidade da microbiota intestinal têm sido implicadas em diversas doenças autoimunes, incluindo aquelas que afetam a glândula tireoide, mas os mecanismos exatos ainda não estão claros, apesar dos avanços recentes na pesquisa. **Objetivo:** Investigar a influência da microbiota intestinal nas respostas imunes da tireoide usando ferramentas bioinformáticas. **Métodos:** O estudo utilizou dados de sequências genômicas e proteômicas do banco de dados NCBI para avaliar a influência da microbiota intestinal nas respostas imunes da tireoide. Foram realizadas análises taxonômicas e metagenômicas, e ferramentas de anotação funcional foram usadas para identificar sequências e vias envolvidas na resposta imune. Redes biológicas foram construídas para entender as interações biológicas complexas. **Resultados:** Um total de 944 sequências de microrganismos específicos para a resposta imune da tireoide foi obtido do NCBI. A composição taxonômica consistiu em *Agathobacter rectalis*, *Bacteroides stercoris*, *Bifidobacterium faecale*, *Clostridium coccoides*, *Clostridium leptum*, *Lactobacillus iners*, *Prevotella jejuni* e *Roseburia*. *Bacteroides stercoris* representou 35% da composição, *Agathobacter rectalis* representou 25%, *Prevotella jejuni* representou 15% e *Roseburia* representou 10%. O índice de diversidade taxonômica foi de 0,85, indicando alta diversidade. Redes biológicas foram construídas, revelando 8 módulos significativos. A via de sinalização NF-kB e MAPK foi

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thyroid immune response. **Conclusions:** We identified a diverse set of microorganisms specific to thyroid immune response, and through biological network analysis we identified a critical signaling pathway in modulating thyroid immune response.

Keywords: Gastrointestinal microbiome, thyroid gland, autoimmune diseases, computational biology.

identificada como a via específica que influencia a microbiota intestinal nas respostas imunes da tireoide, desempenhando um papel crítico na modulação da resposta imune da tireoide. **Conclusão:** Identificamos um conjunto diversificado de microrganismos específicos para a resposta imune da tireoide e, por meio da análise de redes biológicas, identificamos a via de sinalização como sendo essencial na modulação da resposta imune da tireoide.

Descritores: Microbioma gastrointestinal, glândula tireoide, autoimunidade, biologia computacional.

Introduction

In recent years, an increasing body of evidence has highlighted the intricate interplay between gut microbiota and host immune systems. The gut microbiota, a complex ecosystem of microorganisms residing in the intestinal tract, actively influences immune response and plays a crucial role in maintaining overall health and homeostasis.[1,2] Perturbations in the composition and functionality of the gut microbiota have been implicated in various autoimmune diseases, including those affecting the thyroid gland.[3]

Autoimmune thyroid diseases, such as Hashimoto's thyroiditis and Graves' disease, are characterized by dysregulated immune responses targeting self-antigens in the thyroid gland.[4,5] While genetic predisposition and environmental factors contribute to the development of autoimmune thyroid diseases, emerging evidence suggests that gut microbiota may exert a significant influence on the initiation and progression of these disorders.[6] Alterations in the composition and metabolic activities of gut microbiota can affect immune tolerance, leading to a breakdown of self-tolerance mechanisms and subsequent autoimmunity.[7]

Bioinformatics has revolutionized our ability to decipher complex biological systems by integrating and analyzing vast amounts of multi-omics data.[8] In the context of investigating the relationship between microorganisms and their hosts, bioinformatics allows for the comprehensive analysis of microbiome data, the identification of microbial taxa, and the prediction of functional pathways involved in disease pathogenesis.[9] By harnessing the power of bioinformatics, we can unravel the intricate network of interactions between gut microbiota and thyroid immune response.

Despite advancements in our understanding of the gut microbiota's role in health and disease, there remains a notable research gap in the specific relationship between gut microbiota and thyroid-related autoimmune conditions, particularly through bioinformatics analysis. Existing studies have primarily focused on clinical and experimental investigations, often lacking the integrative analysis necessary to comprehensively characterize the intricate interplay between gut microbes and immune response. This gap must be bridged to unlock the potential for personalized treatment strategies targeting the gut microbiota-immune axis in autoimmune thyroid diseases.

This study assessed the relationship between gut microbiota and thyroid immune response by employing bioinformatics tools and approaches.

Methods

Publicly available genomic and proteomic sequence data from the National Center for Biotechnology Information (NCBI) database were used in this study. Sequences related to microorganisms associated with thyroid immune response were collected. Pre-processing steps, including the removal of redundant and low-quality sequences, were performed to ensure data quality. We used the NCBI Taxonomy browser, a powerful tool for identifying the taxonomic origin of sequences, which allowed taxonomic composition to be compared between conditions and groups.

Metagenomic analysis was conducted to investigate the functional aspects of immune response. Proteomic sequences were again collected from the NCBI database and underwent pre-processing. The MG-RAST and MetaPhlAn tools were used to assess taxonomic diversity. Functional annotation tools, such

as KEGG and GO, were used to identify genes and pathways involved in immune response.

To understand the complex biological interactions underlying thyroid immune response, biological networks were constructed using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) and the NCBI Gene databases. Significant modules within these networks were identified utilizing algorithms such as Louvain and Walktrap.

By using these techniques and tools, we aimed to gain insight into the influence of gut microbiota on thyroid immune response, contributing to our understanding of the intricate interplay between the microbiome and the immune system.

According to the Brazilian National Research Ethics Committee (CONEP), this study did not require submission to an ethics committee as it relied on publicly available databases, without involving human or animal subjects.

Results

Taxonomic Composition

– A total of 944 sequences of microorganisms specific to thyroid immune response were obtained from the NCBI Taxonomy Browser: *Agathobacter rectalis* (260 Genomes), *Bacteroides stercoris* (164 Genomes), *Bifidobacterium faecale* (1 Genome), *Clostridium coccoides* (25 Genomes), *Clostridium leptum* (31 Genomes), *Lactobacillus iners* (381 Genomes), *Prevotella jejuni* (22 Genomes), and *Roseburia* (59 Genomes) (Table 1).

– The taxonomic variation in microorganism genera specific to thyroid immune response was: *Bacteroides stercoris* (35%), *Agathobacter rectalis*

Table 1
National Center for Biotechnology Information taxonomy browser results for specific microorganisms

Species	***Agathobacter rectalis***	***Bacteroides stercoris***	***Bifidobacterium faecale***	***Clostridium coccoides***
Domain	Bacteria	Bacteria	Bacteria	Bacteria
Phylum	Firmicutes	Bacteroidetes	Actinobacteria	Firmicutes
Class	Clostridia	Bacteroidia	Actinobacteria	Clostridia
Order	Clostridiales	Bacteroidales	Bifidobacteriales	Clostridiales
Family	Lachnospiraceae	Bacteroidaceae	Bifidobacteriaceae	Clostridiaceae
Genus	Agathobacter	Bacteiroides	Bifidobacterium	Clostridium

Species	***Clostridium leptum***	***Lactobacillus iners***	***Prevotella jejuni***	***Roseburia***
Domain	Bacteria	Bacteria	Bacteria	Bacteria
Phylum	Firmicutes	Firmicutes	Bacteroidetes	Firmicutes
Class	Clostridia	Bacilli	Bacteroidia	Clostridia
Order	Clostridiales	Lactobacillales	Bacteroidales	Clostridiales
Family	Ruminococcaceae	Lactobacillaceae	Prevotellaceae	Lachnospiraceae
Genus	Clostridium	Lactobacillus	Prevotella	Roseburia

(25%), *Prevotella jejuni* (15%), *Roseburia* (10%), other (15%).

Taxonomic Diversity

– Metagenomic analysis revealed significant taxonomic diversity.

– To calculate the taxonomic diversity index, the Shannon-Wiener index formula was used: $H' = -\sum (pi * \ln(pi))$, and the proportions of each species relative to the total species count were as follows: *Agathobacter rectalis*: 260/1,768,139 ≈ 0.000147, *Bacteroides stercoris*: 164/1,768,139 ≈ 0.000093, *Bifidobacterium faecale*: 1/1,768,139 ≈ 0.000001, *Clostridium coccoides*: 25/1,768,139 ≈ 0.000014, *Clostridium leptum*: 31/1,768,139 ≈ 0.000018, *Lactobacillus iners*: 381/1,768,139 ≈ 0.000215, *Prevotella jejuni*: 22/1,768,139 ≈ 0.000012, and *Roseburia*: 59/1,768,139 ≈ 0.000033.

– The taxonomic diversity index was high (0.85).

Construction of Biological Networks

– Integration of databases (STRING) allowed the construction of biological networks.

– Result: A total of 8 significant modules were identified in the networks.

Signaling Pathway Obtained from the Study

– The NF-kB and MAPK signaling pathway was identified as specifically influencing gut microbiota (Figure 1).

– Activation of the NF-Kb and MAPK pathway regulates immune response by triggering inflammation and adaptive immune genes.

– The NF-kB and MAPK signaling pathway plays a critical role in modulating thyroid immune response, highlighting the importance of the interaction between the microbiome and the immune system in thyroid function.

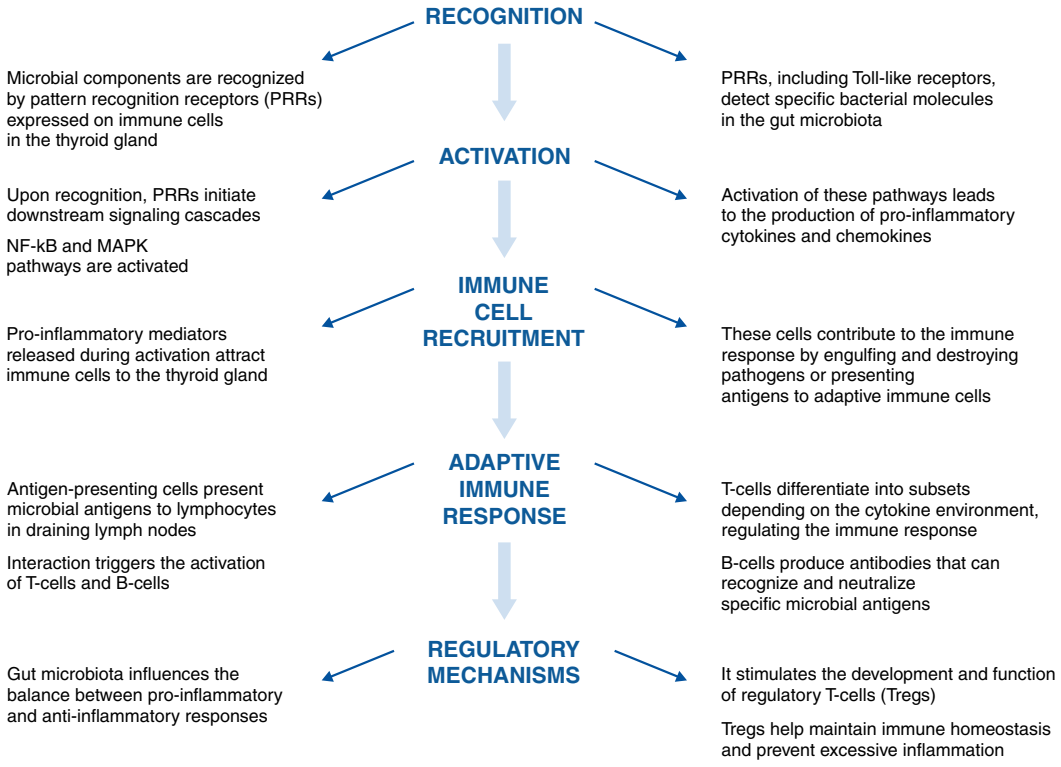

Figure 1
Signaling pathway for thyroid immune response

Discussion

Our results provide insight into the taxonomic composition, diversity, and functional aspects of microorganisms associated with thyroid immune response.

The taxonomic composition analysis demonstrated that various microorganisms are specific to thyroid immune response. The dominant microorganisms in the database evaluated in this study were *Agathobacter rectalis*, *Bacteroides stercoris*, *Lactobacillus iners*, and *Roseburia*. Our findings are consistent with previous studies reporting the presence of these microbiota genera in the gut.[10,11] However, the proportions of each microorganism differed among these studies, indicating potential variations influenced by factors such as geographical location, diet, and health status. It is worth noting that *Bifidobacterium faecale* and *Prevotella jejuni* were also identified, which have not been extensively studied in the context of thyroid immune response.

The taxonomic diversity of microorganisms associated with thyroid immune response was also investigated, indicating a high level of diversity among the microorganisms present in thyroid immune response. This is in line with previous studies that have reported gut microbiota diversity.[12,13] The Shannon-Wiener index, which accounts for both the richness and evenness of species, is used to quantify taxonomic diversity.[14] Our results indicated a high level of diversity among the studied microorganisms. This suggests that the gut microbiota could affect thyroid immune response through a complex network of interactions. This finding is consistent with previous studies that have reported diverse microbial communities in various body sites and their correlation with immune response.[15] The high taxonomic diversity suggests that multiple microorganisms contribute to the regulation of thyroid immune response, potentially influencing overall immune system functionality.

Our understanding of the interactions between microorganisms involved in thyroid immune response was deepened by integrating databases, constructing biological networks that provided insight into the potential interactions between the identified microorganisms. The significant modules found in the networks indicate the presence of interconnected microorganisms that may work together to influence thyroid immune response. The identification of significant modules within these networks highlight the complex and interconnected nature of microorganism function.[16] This finding is in line with previous research that has emphasized the importance of studying microbial interactions and community dynamics in various biological systems.[17] Thus, biological network construction can be a valuable tool for further investigating the specific roles of individual microorganisms and their interactions in thyroid immune response.

We also found that the NF-kB and MAPK signaling pathway influences gut microbiota in thyroid immune response. Activation of these pathways plays a critical role in the immune system by triggering inflammation and adaptive immune response.[18] This finding supports previous literature that has demonstrated the impact of microbial-host interactions in immune signaling pathways.[19,20] The identification of this signaling pathway further emphasizes the importance of the interaction between the gut microbiome and the immune system in thyroid regulation.

The analysis of taxonomic composition and diversity provided valuable insight into the microorganisms associated with thyroid immune response. The identification of specific microorganisms, the assessment of taxonomic diversity, the construction of biological networks, and the identification of key signaling pathways contribute to our understanding of the complex interactions between microorganisms and the immune system in thyroid regulation.[21] Thus, by comparing our findings with the literature, we can better comprehend the significance of the microbial-host interactions in the context of thyroid immune response.

Conclusions

We identified a diverse set of microorganisms specific to thyroid immune response, and through biological network analysis we identified a critical signaling pathway in thyroid immune response modulation.

References

1. Belkaid Y, Hand TW. Role of the microbiota in immunity and inflammation. Cell. 2014;157(1):121-41.
2. Hand T, Belkaid Y. Microbial control of regulatory and effector T cell responses in the gut. Curr Opin Immunol. 2010;22(1):63-72.
3. Virili C, Fallahi P, Antonelli A, Benvenga S, Centanni M. Gut microbiota and Hashimoto's thyroiditis. Rev Endocr Metab Disord. 2018;19(4):293-300.

4. Weetman AP. An update on the pathogenesis of Hashimoto's thyroiditis. J Endocrinol Invest. 2021;44(5):883-90.
5. Kahaly GJ. Management of Graves Thyroidal and Extrathyroidal Disease: An Update. J Clin Endocrinol Metab. 2020;105(12):3704-20.
6. Zhao H, Yuan L, Zhu D, Sun B, Du J, Wang J. Alterations and Mechanism of Gut Microbiota in Graves' Disease and Hashimoto's Thyroiditis. Pol J Microbiol. 2022;71(2):173-89.
7. Kriegel MA. Self or non-self? The multifaceted role of the microbiota in immune-mediated diseases. Clin Immunol. 2015;159(2):119-21.
8. Nobile MS, Cazzaniga P, Tangherloni A, Besozzi D. Graphics processing units in bioinformatics, computational biology and systems biology. Brief Bioinform. 2017;18(5):870-85.
9. Pallen MJ. Microbial bioinformatics 2020. Microb Biotechnol. 2016;9(5):681-6.
10. Sittipo P, Lobionda S, Lee YK, Maynard CL. Intestinal microbiota and the immune system in metabolic diseases. J Microbiol. 2018;56(3):154-62.
11. Gul L, Modos D, Fonseca S, Madgwick M, Thomas JP, Sudhakar P, et al. Extracellular vesicles produced by the human commensal gut bacterium Bacteroides thetaiotaomicron affect host immune pathways in a cell-type specific manner that are altered in inflammatory bowel disease. J Extracell Vesicles. 2022;11(1):e12189.
12. Sender R, Fuchs S, Milo R. Revised Estimates for the Number of Human and Bacteria Cells in the Body. PLoS Biol. 2016;14(8):e1002533.
13. Zhao F, Feng J, Li J, Zhao L, Liu Y, Chen H, et al. Alterations of the Gut Microbiota in Hashimoto's Thyroiditis Patients. Thyroid. 2018;28(2):175-86.
14. Shannon CE. A mathematical theory of communication. Bell System Technical Journal. 1948;27:379-423.
15. Rooks MG, Garrett WS. Gut microbiota, metabolites and host immunity. Nat Rev Immunol. 2016;16(6):341-52.
16. Guven-Maiorov E, Tsai CJ, Nussinov R. Structural host-microbiota interaction networks. PLoS Comput Biol. 2017;13(10):e1005579.
17. Cordero OX, Datta MS. Microbial interactions and community assembly at microscales. Curr Opin Microbiol. 2016;31:227-34.
18. Peng C, Ouyang Y, Lu N, Li N. The NF-κB Signaling Pathway, the Microbiota, and Gastrointestinal Tumorigenesis: Recent Advances. Front Immunol. 2020;11:1387.
19. Tripodi D, Conti F, Rosati M, Maccauro G, Saggini A, Cianchetti E, et al. IL-36 a new member of the IL-1 family cytokines. J Biol Regul Homeost Agents. 2012;26(1):7-14.
20. Yang M, Sun B, Li J, Yang B, Xu J, Zhou X, et al. Alteration of the intestinal flora may participate in the development of Graves' disease: a study conducted among the Han population in southwest China. Endocr Connect. 2019;8(7):822-8.
21. Cuan-Baltazar Y, Soto-Vega E. Microorganisms associated to thyroid autoimmunity. Autoimmun Rev. 2020;19(9):102614.

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