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Research Article
In silico Analysis of Tyrosine Kinases Receptor in Papillary and Medullary Thyroid Cancer Using Sequence-alignment-based Methods

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Abstract

Background and Objective: Papillary and medullary thyroid cancers are prevalent. Effective cancer therapies need molecular target identification. Tyrosine kinases regulate cell growth, differentiation and survival. This study sequence-aligns papillary and medullary thyroid cancer tyrosine kinase receptors *in silico*. The investigation seeks tyrosine kinase receptor mutations and structural alterations that may cause these cancers. **Materials and Methods:** Phylogeny estimation was performed for tyrosine kinase proteins from 4 different types of cancer: Thyroid carcinoma, ovary, lung and hepatic cancer. Analyzed the protein domains present in each cancer type and found that the catalytic domain of the protein tyrosine kinases was the predominant domain present in all of the investigated sequences. **Results:** The present results displayed that almost the sequence from cancerous thyroid tissue and other organs did not differ from each other and revealed high similarity. The analysis indicated that the catalytic domain of the protein tyrosine kinases is the predominant domain. **Conclusion:** This information could be used to develop new diagnostic tools and targeted therapies for these types of cancer. Overall, this study provides valuable insights into the molecular mechanisms underlying cancer development and highlights the importance of understanding the protein domains present in different types of cancer.

Key words: Papillary thyroid cancer, medullary thyroid cancer, *in silico*, protein kinase, sequence alignment

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Protein sequences from a wide range of species have flooded the area of Bioinformatics as a consequence of proteome study. In this context, the "proteome" is the set of proteins that are analogous to the genome[1]. The functional characterization is not reflected in the bulk of the predicted protein sequences. As a required step toward integrated organismal study at the gene, transcript, protein and functional levels, the goal is to provide statistical and comparative analysis, in addition to structural and other information for these sequences. This will be accomplished with the help of these sequences[2]. Worldwide, the number of new cases of thyroid cancer has been steadily climbing for decades[3]. All age groups are affected, however, younger women (those under 45) are at a somewhat higher risk[4]. When it comes to differentiated thyroid cancers, papillary thyroid carcinoma is the most prevalent kind[5]. The aberrant activation of receptor tyrosine kinases, often known as RTKs, is a factor that contributes to the transformation, proliferation and metastasis of human cancers[6,7]. The expression, conformation and/or stability of tyrosine kinases can be altered by mutations, which typically leads to the activation of the signaling pathways in a constitutive manner[8]. Mutations have varied consequences on many biological proteins because tyrosine kinases are important components of signaling cascades[9]. Over the past 20 years, a variety of molecules that target Receptor Tyrosine Kinases (RTKs) have been employed in the field of oncology as either primary or secondary treatments for various forms of cancer. Nonetheless, the efficacy of these interventions is constrained by the emergence of resistance or untoward reactions[10].

Moreover, the tyrosine kinases receptor is a family of receptors that are located in the body's cells. They are responsible for the activity of tyrosine proteins, which in turn are responsible for the body's immune system[11]. The receptor is located in the papillary thyroid cancer and medullary thyroid cancer cells and it helps to interpret and process the signals sent from the cells[12]. The sequence of the receptor is important in determining its activity. It is located in the papillary thyroid cancer cells and it helps to interpret the signals sent from the cells.

The present investigation aims to provide a comprehensive overview of the principal attributes of Receptor Tyrosine Kinases (RTKs) in two distinct types of thyroid cancer, namely papillary and medullary, as well as certain visceral cancers. This will be accomplished through the utilization of sequence alignment techniques, assessment of the physicochemical properties of amino acid sequences and comparison of protein domains.

MATERIALS AND METHODS

Study area: The current study was conducted at Biology Department, Jazan University, from February till April, 2023.

Study design: The study is an *in silico* analysis, which means that it is conducted using computational methods rather than experimental methods. The researchers used sequence-alignment based methods to analyze the tyrosine kinase receptors in papillary and medullary thyroid cancer. Specifically, they used Jalview, WebLogo and MEGA software for the analysis.

Jalview is a software tool used for the analysis of multiple sequence alignments. The researchers likely used Jalview to align the sequences of tyrosine kinase receptors in papillary and medullary thyroid cancer and to identify similarities and differences between the sequences.

WebLogo is a software tool used for the visualization of sequence logos, which represent the frequencies of amino acids at each position in a sequence alignment. The researchers likely used WebLogo to create sequence logos of the tyrosine kinase receptors in papillary and medullary thyroid cancer, which can provide insights into the conservation of specific amino acid residues and potential functional domains.

Molecular Evolutionary Genetics Analysis (MEGA) version 10 is a software tool used for the analysis of molecular evolutionary genetics data. The researchers likely used MEGA to construct a phylogenetic tree of the tyrosine kinase receptors in papillary and medullary thyroid cancer, which can provide insights into the evolutionary relationships between the sequences.

Sequences, alignment and construction of the phylogenetic tree: Protein sequences for the tyrosine-protein kinase receptor of (papillary and medullary) thyroid tumor samples as well as other tissues were obtained from NCBI (Genbank and dbEST), UniProtKB and UniProtKB database. The source and accession numbers of the corresponding entries were listed in Table 1.

Protein multiple sequence alignment was undertaken so that evolutionary linkages could be discovered, which would then allow common patterns to be identified[13]. Multiple alignments of sequences were performed. Software update to version 2.8 for Jalview. WebLogo was used to build sequence logos, which were then used to construct graphical representations of the tyrosine-protein kinase amino acid patterns found inside a multiple sequence alignment[14]. Phylogenetic trees were generated with the help of MEGA by utilizing the Neighbor-Joining Method[15,16]. The model building

Table 1: Accession numbers of NCBI entries of tyrosine kinases receptor from cancerous thyroid tissue and other organs (*Homo sapiens*)

Tissue	Accession GenBank	aa	DB Source
Papillary thyroid cancer	P07949	1114	UniProtKB
Papillary thyroid cancer	NP_001371955	1746	UniProtKB
Medullary thyroid cancer	NP_001393707	830	REFSEQ
Medullary thyroid cancer	NP_001393709	815	REFSEQ
Medullary thyroid cancer	NP_001393703.1	897	REFSEQ
Medullary thyroid cancer	NP_001393697.1	984	REFSEQ
Papillary thyroid cancer	NP_001012338	839	REFSEQ
Papillary thyroid cancer	NP_001362741.1	817	REFSEQ
Papillary thyroid cancer	NP_001362740.1	825	REFSEQ
Hepatic cancer	P08581	1390	UniProtKB
Ovarian cancer cells	NP_005415.1	1138	REFSEQ
Lung cancer	Q9UM73	1620	UniProtKB

aa: Arrangement of amino acids in a protein

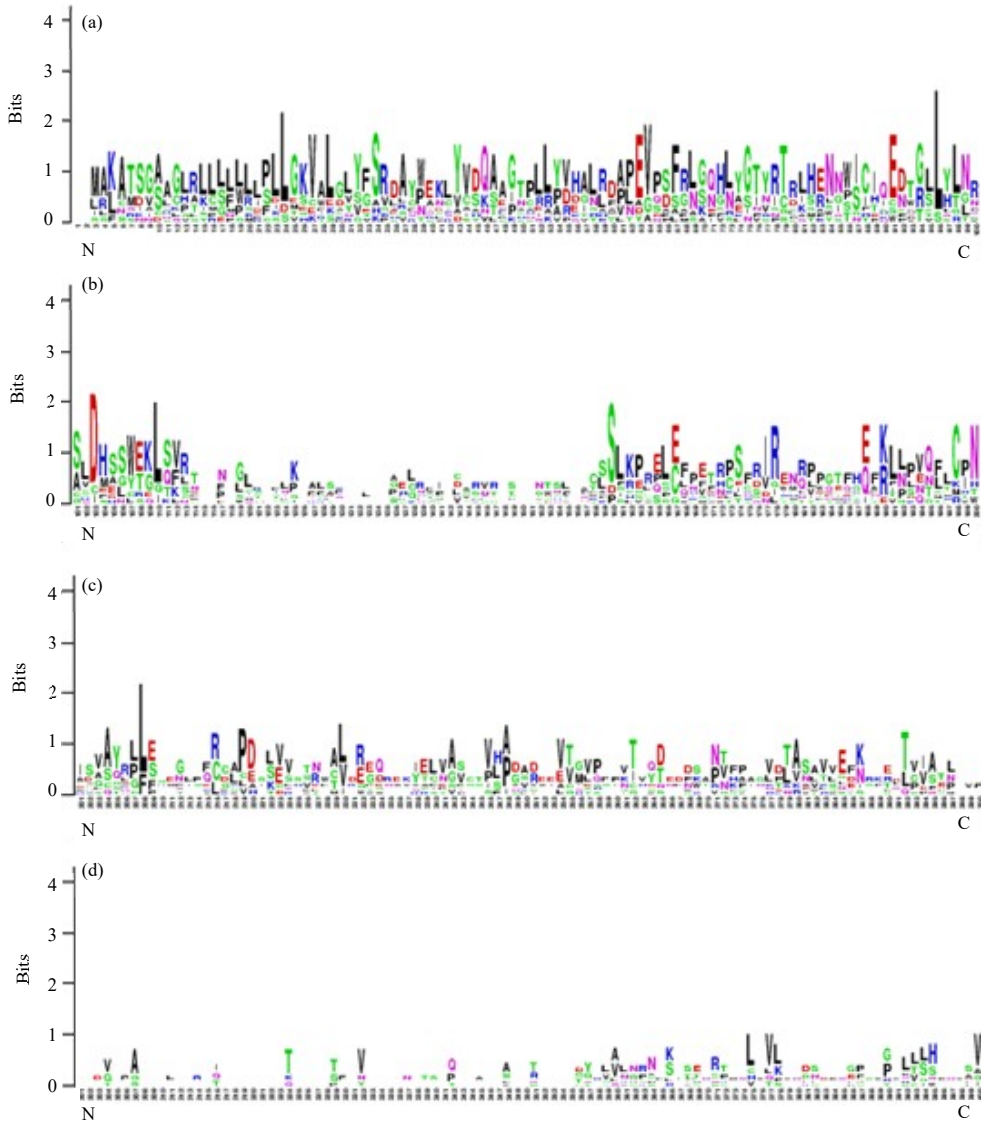

Fig. 1(a-d): Graphical depiction of a scoring matrix developed for multiple sequence alignments of tyrosine kinases protein from thyroid carcinoma and other tissues. A score to each position in the sequence is assigned based on the frequency with which each of the 20 most common amino acids in proteins appears in alignment sequence (1-400 aa)

Jones-Taylor-Thornton was used to produce a distance matrix. Displayed in accordance with the approach given by Tom Schneider and Mike Stephens was a graphical means of describing and visualizing consensus data created of amino acid multiple sequence alignments developed.

Matthews Coefficient calculation: The Matthews Coefficient is a measure of the packing density of protein molecules in a crystal. It is also sometimes called the solvent content of a crystal because it estimates how much of a crystal's volume is occupied by solvent. The Matthews Coefficient is calculated according to Kantardjieff and Rupp[17] as follows:

$$\text{Matthews Coefficient} = \frac{\text{V (Volume of the protein molecule in cubic Å)}}{\text{N (Number of amino acid residues)}}$$

RESULTS

Sequence logos for protein molecules: Each letter in the logo represents a stack in the sequence. Each stack's height (in bits) represents the degree of sequence conservation at that place, whereas the height of individual symbols inside the stack reflects the frequency with which the relevant amino or nucleic acid appears at that position as shown in Fig. 1. The sequence logo of tyrosine kinase receptors generally indicates that the sequences derived from cancerous thyroid tissue and other organs exhibit a high degree of similarity with minimal differences.

Concerning the phylogeny of tyrosine kinase proteins from thyroid cancer and other tissues. Mega 10.1.8 was used to create a Neighbor-Joining tree. The multiple sequence alignments were depicted in Fig. 2. A constructed version of the phylogenetic tree is shown in Fig. 3. According to the current findings, a Neighbor-Joining tree of tyrosine kinases protein from papillary and medullary thyroid carcinoma provides a comparison of the amino acid sequences of tyrosine kinases protein from different organs such as the liver, the lung and the ovary. The findings indicate that the phylogenetic analysis is composed of three distinct groups (Fig. 3). In cluster 1 papillary and medullary thyroid cancer is related to ovarian cancer. In cluster 2 medullary thyroid cancer is related to lung cancer only. However, cluster 3 represents that papillary thyroid cancer is related to hepatic cancer.

Fig. 2: Multiple sequence alignment of tyrosine kinases protein from thyroid carcinoma and other tissues

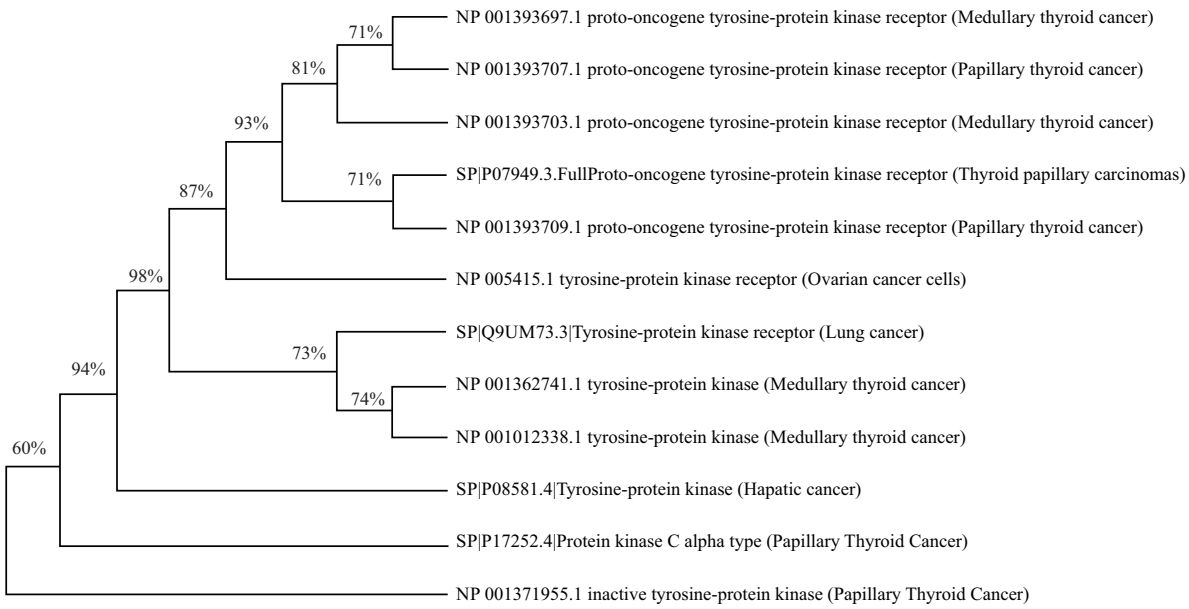

Fig. 3: Protein sequences from several tissues, including thyroid cancer, were analyzed for their phylogenetic relationships. Each node in the tree represents a percentage value acquired from resampling the data set 100 times using the Bootstrap method. Using the Jones-Taylor-Thornton model to estimate pairwise distances, the Neighbor-Join and BioNJ algorithms were applied to produce the resulting tree

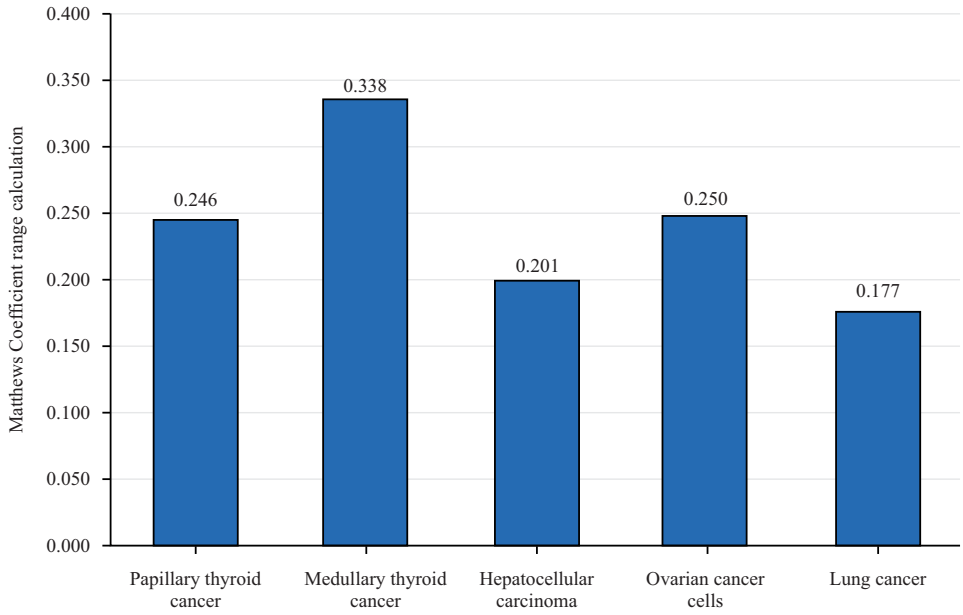

Fig. 4: Matthews Coefficient for tyrosine kinases protein from thyroid carcinoma and other tissues

Matthews Coefficient, the current results revealed that the highest value scored for medullary thyroid cancer (0.338) followed by ovarian then papillary thyroid cancer as shown in Fig. 4.

The protein domains in different cancer types. The current analysis indicated that the catalytic domain of the protein tyrosine kinases is the predominant domain present in the investigated sequences as shown in Fig. 5.

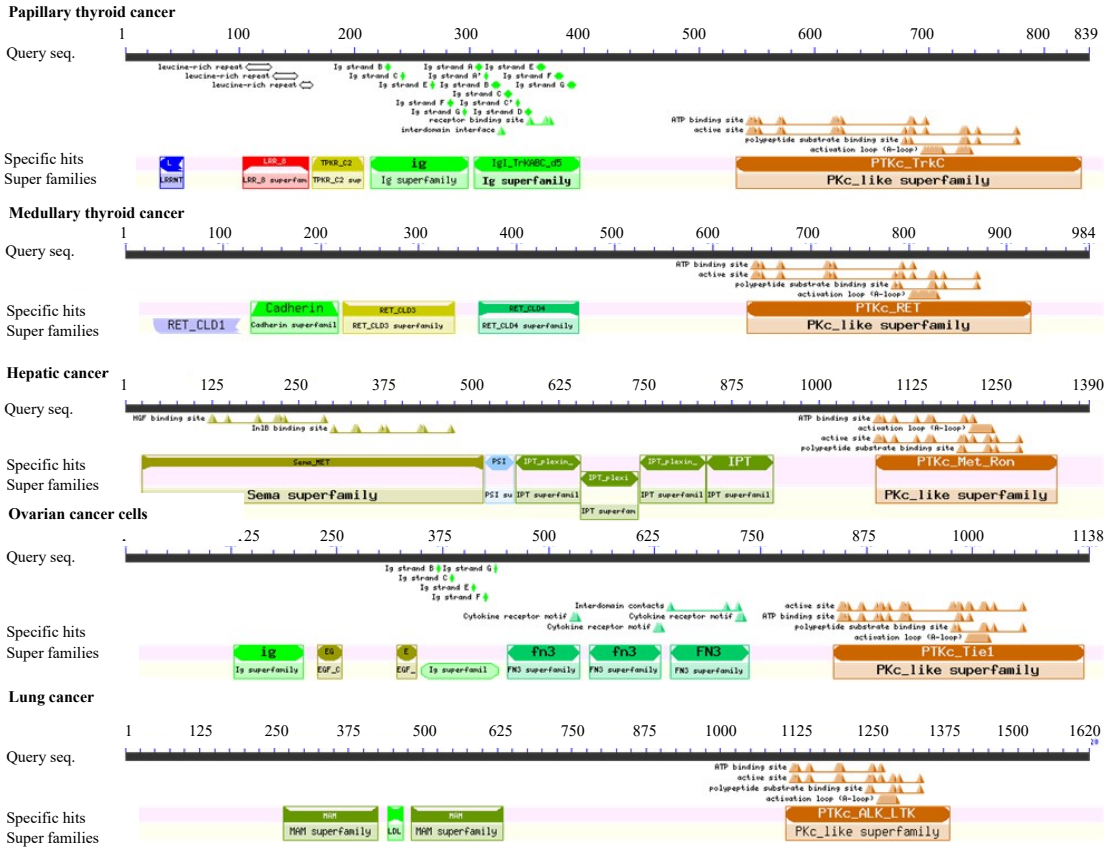

Fig. 5: Functional classification of proteins via subfamily domain architectures of tyrosine kinases protein from thyroid carcinoma and other tissues

DISCUSSION

Current results provide important insights into the molecular characteristics of tyrosine kinase receptors in papillary and medullary thyroid cancer. The Matthews Coefficient analysis revealed that the highest value was scored for medullary thyroid cancer, followed by ovarian and papillary thyroid cancer. This suggests that there are differences in the amino acid sequences of tyrosine kinase receptors between these types of cancer, which may contribute to their distinct molecular characteristics and clinical outcomes. The Neighbor-Joining tree of tyrosine kinases protein from papillary and medullary thyroid carcinoma provides a comparison of the amino acid sequences of tyrosine kinases protein from different organs such as the liver, the lung and the ovary. The findings indicated that the tyrosine kinase receptors in thyroid cancer are closely related to those in other organs, which may have implications for the development of targeted therapies for thyroid cancer. Overall, these results suggest that further investigation into the molecular characteristics of tyrosine kinase receptors in thyroid cancer is warranted. This may involve the identification of specific mutations or structural alterations in these receptors that contribute to the development and progression of thyroid cancer, as well as the development of targeted therapies that can selectively inhibit these receptors.

Receptor Tyrosine Kinases (RTKs) play a crucial role in the pathogenesis of thyroid cancer, specifically papillary and medullary thyroid cancer[18]. Papillary thyroid cancer (PTC) is the most common type of thyroid cancer and its development is associated with several genetic alterations, including mutations in the RET proto-oncogene and BRAF oncogene[19]. These mutations lead to the activation of RTK signaling, which promotes cell proliferation, angiogenesis andinvasion[20]. Medullary thyroid cancer (MTC) arises from the calcitonin-producing C-cells of the thyroid gland and is associated with mutations in RET[21]. The RET gene encodes a transmembrane RTK that is activated upon binding to its ligands, leading to downstream signaling that promotes cell growth and survival[22].

In both PTC and MTC, the activation of RTK signaling provides a therapeutic target for the treatment of these cancers[23]. The development of small molecule inhibitors targeting RTKs, such as vandetanib and lenvatinib, has shown promising results in clinical trials for both PTC and MTC[24].

Multiple sequence alignment (MSA) is a powerful tool used to identify shared patterns and relationships between amino acid sequences[25] . In this context, MSA of Receptor Tyrosine Kinases (RTKs) could provide insight into potential similarities and differences between RTKs involved in papillary and medullary thyroid cancers.

The MSA of RTKs in thyroid cancer has been performed in several studies. For example, a study published in the Best Practice and Research Clinical Endocrinology and Metabolism, used MSA to analyze the sequences of RET, a key RTK implicated in medullary thyroid cancer. The authors identified several conserved motifs in the extracellular domain of RET that were shared between different species, suggesting that these motifs play an important role in the regulation of RET function[26].

Similarly, a study published in Thyroid used MSA to compare the sequences of RTKs implicated in papillary thyroid cancer, including RET and BRAF. The authors identified several conserved motifs in the kinase domains of these RTKs, which are thought to be important for catalytic activity and downstream signaling[27].

Phylogenetic tree analysis of protein sequences is the process of constructing a branching diagram that represents the evolutionary relationships between different species or groups of organisms, based on their similarities and differences in protein sequences. Protein sequences are the linear arrangement of amino acids that make up proteins, which are the building blocks of life[28]. Phylogenetic tree analysis of protein sequences typically involves three main steps: Sequence alignment, phylogenetic inference and tree visualization. In the first step, multiple protein sequences from different species or groups are aligned to identify the positions of homologous (similar) amino acids. This alignment allows for the identification of conserved regions, which are evolutionarily important and provide information about the relatedness of the organisms being studied[29]. In the second step, phylogenetic inference methods are used to construct a tree that reflects the evolutionary history of the organisms based on their protein sequences. Different methods of inference include Maximum Parsimony, Maximum Likelihood and Bayesian Inference, which use different algorithms and models to estimate the most likely evolutionary relationships[30]. In the final step, the tree is visualized, usually in the form of a branching diagram or cladogram. The branches represent the evolutionary relationships between organisms, with the length of the branches indicating the amount of evolutionary change that has occurred. The clustering of taxa on the tree can also indicate the degree of similarity or divergence in their protein sequences. The current results revealed that both papillary and medullary thyroid cancer is related to ovarian cancer. The present results are in agreement with Brogioni *et al.*[31], who stated that papillary thyroid carcinoma metastasize to the ovary, combined with an autoimmune thyroiditis. Moreover, Cosci *et al.*[32] declared that in 98% of hereditary and 40% of sporadic medullary thyroid carcinomas, germline and somatic RET oncogene mutations are detected.

Proteins contain domains, which are structurally and functionally independent units. Their amino acid sequence and three-dimensional structure can be used to identify the functions they perform. Protein sequence similarity, on the other hand, is a measure of how similar two protein sequences are in terms of their amino acid composition and order[33]. Comparing protein domains to protein sequence similarity is like comparing a set of specialized tools to a general-purpose tool. While protein domains are highly specific and can provide detailed information about a protein's function, sequence similarity analysis can reveal broader patterns and relationships between different proteins[34]. Overall, both protein domains and sequence similarity analysis are valuable tools for understanding protein structure and function. By combining these approaches, scientists can gain a more complete picture of the complex world of proteins and their interactions within cells and organisms.

Targeted therapy is a type of cancer treatment that involves the use of drugs that specifically target molecular or genetic abnormalities that drive the growth and survival of cancer cells[35]. Receptor Tyrosine Kinases (RTKs) have become important targets for cancer therapy due to their role in promoting cell proliferation, angiogenesis and invasion. Some of the targeted therapies work by inhibiting RTKs in cancer[36]. Small molecule inhibitors are drugs that bind to the ATP-binding site of the kinase domain of RTKs, preventing them from phosphorylating downstream targets. Examples of small molecule inhibitors that target RTKs include imatinib (targeting BCR-ABL in chronic myelogenous leukemia), gefitinib and erlotinib (targeting EGFR in non-small cell lung cancer) and sunitinib and sorafenib (targeting multiple RTKs in renal cell carcinoma[37]). Monoclonal antibodies are antibodies that target specific extracellular domains of RTKs, preventing ligand binding and activation. Examples of monoclonal antibodies that target RTKs include trastuzumab (targeting HER2 in breast cancer),

bevacizumab (targeting VEGF in multiple solid tumors) and cetuximab and panitumumab (targeting EGFR in colorectal cancer)[38]. Bispecific antibodies are antibodies that simultaneously target two different proteins, such as RTKs and immune cells. Examples of bispecific antibodies that target RTKs include blinatumomab (targeting CD19 in B-cell acute lymphoblastic leukemia) and catumaxomab (targeting EpCAM in malignant ascites)[39]. Recent studies have shown that Receptor Tyrosine Kinases (RTKs) play a crucial role in the development and progression of thyroid cancer, particularly papillary and medullary thyroid cancer. These RTKs are involved in various signaling pathways that regulate cell growth, differentiation and survival[40,41]. One of the most promising findings is that the sequence of RTKs in papillary and medullary thyroid cancer is highly similar. This means that drugs that have been approved for one type of cancer may also be effective in treating the other type[42].

Proteins with high Matthews Coefficients are dense, tightly packed structures, while those with low coefficients are more loosely packed. Matthews Coefficients can be useful in crystallography, as they can help determine suitable conditions for crystallizing proteins and can aid in designing optimal crystallization conditions[43].

The RTKs have become important targets for cancer therapy due to their role in promoting cancer cell growth and survival. The development of small molecule inhibitors, monoclonal antibodies and bispecific antibodies that target RTKs have shown promising results in the treatment of many different types of cancer.

The study has several implications, applications, recommendations and limitations, which are discussed. The study provides insights into the molecular mechanisms and genetic alterations that drive thyroid cancer development and progression. The identification of specific tyrosine kinase receptors that are differentially expressed in papillary and medullary thyroid cancer could lead to the development of targeted therapies for these types of cancer. The study highlights the importance of using computational methods to analyze large datasets and identify potential therapeutic targets.

The findings of this study could inform the development of new drugs or repurposing of existing drugs to target specific tyrosine kinase receptors in thyroid cancer. The computational methods used in this study could be applied to other types of cancer or genetic diseases to identify potential therapeutic targets, or biomarkers. The study recommends further investigation of the identified tyrosine kinase receptors using experimental methods such as cell culture and animal models to validate the computational findings. The study also recommends screening larger cohorts of thyroid cancer patients to confirm the differential expression of the identified tyrosine kinase receptors.

The study is based on *in silico* analysis and does not include experimental validation. The sample size is relatively small, which could limit the generalizability of the findings. The study only focuses on papillary and medullary thyroid cancer and the findings may not apply to other types of thyroid cancer or cancer in general.

CONCLUSION

The RTK inhibitors hold significant potential in the treatment of thyroid cancers, particularly papillary and medullary thyroid cancers. Further research is needed to improve the effectiveness of these treatments and to identify additional novel RTK targets for therapy. Overall, MSA of RTKs can provide valuable insights into the similarities and differences between RTKs involved in different types of thyroid as well as other visceral organ cancer. This information can inform the development of targeted therapies for these cancers, as well as aid in the identification of novel RTKs and potential therapeutic targets.

SIGNIFICANCE STATEMENT

Proteomics analysis is an important tool for understanding the molecular mechanisms underlying cancer development and for identifying potential targets for cancer therapy. By studying the tyrosine kinases receptor in different types of cancer, the present results revealed a similarity between medullary thyroid cancer and lung cancer and a similarity between papillary thyroid cancer and hepatic cancer.

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