Section 3. Preventive Medicine

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UNCOVERING THE GENETIC BASIS OF THYROID CANCER: A STUDY OF SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS)

Abstract. Thyroid cancer remains a significant public health concern, with an estimated 43.720 new cases predicted in 2023, affecting both men and women. Our study aimed to explore the genetic underpinnings of thyroid cancer predisposition by analyzing Single Nucleotide Polymorphisms (SNPs). To do so, we leveraged Genome-Wide Sequencing data from the Sequence Read Archive and developed tailored analysis pipelines using Bowtie 2, a popular alignment tool, to map the sequences onto chromosome 7 and perform variant calling. Our analysis revealed 9 SNPs that were present in over 90% of thyroid cancer patients but not in the normal population. These findings hold promise for the development of new strategies for the early detection and prevention of thyroid cancer.

Keywords: thyroid, cancer, SNPs.

Introduction

Cancer

In 2023, thyroid cancer continued to be a significant health challenge in the United States, with The American Cancer Society reporting 43.720 new cases (12.540 in men and 31.180 in women) and 2,120 deaths (970 men and 1,150 women) (American Cancer Society, 2021). This cancer results from the uncontrolled growth of cells in the thyroid gland, and it can be classified into four types: papillary, follicular, medullary, and anaplastic. Among them, papillary carcinoma is the most common, accounting for about 80% of cases, and has a slow-growing nature with the best prognosis among all thyroid cancer types (*Thyroid Cancer* – Patient Version – *NCI*, n.d.). Even though it can spread to nearby lymph nodes, papillary carcinoma rarely causes life-threatening complications. Being aware of the type of thyroid cancer is essential for choosing the most effective treatment and improving the chances of recovery.

Following papillary carcinoma, follicular thyroid cancer is the second most common type, accounting for about 15% of cases. Although it has a good prognosis, follicular cancer is more aggressive than papillary cancer and is more likely to spread to other organs, such as the lungs and bones, even if it does not spread to the nearby lymph nodes. Medullary thyroid cancer, the third most common type, represents 4% of cases and is less differentiated than papillary and follicular cancers. This type of cancer may also spread to the lymph nodes and other organs, and high levels of calcitonin and carcinoembryonic antigen can be indicative of its presence (*Thyroid Cancer* – Patient Version – *NCI*, n.d.). Anaplastic thyroid cancer is the rarest form, making up only 2% of all cases, but it is also the most aggressive type. This cancer grows rapidly, is highly undifferentiated, and can quickly spread to other parts of the body. Understanding the characteristics of each type of thyroid cancer is crucial to determine the appropriate diagnosis, treatment, and

management strategies for patients (*Thyroid Cancer* – Patient Version – *NCI*, n.d.).

Stages

Thyroid cancer is staged based on the extent of metastasis. Stage I is when the cancer is limited to the thyroid gland and has not spread beyond it (Thyroid Cancer – Patient Version – NCI, n.d.). Stage II is characterized by tumor growth and appearance in surrounding tissues (Thyroid Cancer – Patient Version – NCI, n.d.). In Stage III, cancer has spread to nearby lymph nodes within level VI, as shown in Figure 3. Stage IV is the most advanced stage, in which cancer has spread to distant sites outside of the level VI lymph node and may involve gross soft tissue extension (Thyroid Cancer – Patient Version – NCI, n.d.). Please refer to Figures 1–3 for visual representations of the different stages of thyroid cancer.

Table 1: Pathological TNM criteria

	Thyroid carcinoma
STAGE 1	Less than 2 cm in diameter without evidence of disease outside of the thyroid gland.
STAGE 2	Between 2 and 4 cm without evidence of extra thyroidal disease.
STAGE 3	Greater than 4 cm, or level VI nodal metastases or microscopic extra thyroidal invasion regardless of tumor size.
STAGE 4	Any distant metastases, or lymph node involvement outside of level VI, or gross soft tissue extension.

Figure 1. The stages of thyroid cancer, based on the degree of metastasis. Stage 1 represents cancer limited to the thyroid gland, while stage 4 indicates cancer has spread to distant areas. As cancer progresses through each stage, the likelihood of metastasis and the severity of cancer increases (Saeed et al. [15])

Treatment

There are several treatment options available for thyroid cancer. The six standard treatments are thyroid surgery, radiation therapy, chemotherapy, thyroid hormone therapy, targeted drug therapy, and watchful waiting (National Cancer Institute [7]). The type of treatment recommended will depend on various factors, such as the stage and type of thyroid cancer, as well as the patient's age and overall health.

Surgical options for thyroid cancer include thyroidectomy, thyroid lobectomy, and lymph node dissection. Thyroidectomy involves the removal of all or most of the thyroid gland, while thyroid lobectomy involves the removal of only a portion of the gland. In some cases, individuals who have inherited a gene that is likely to cause thyroid cancer may choose to have a thyroidectomy to decrease their chance of developing medullary thyroid cancer (National Cancer Institute [7]). Lymph node dissection involves the removal of lymph nodes in the neck area. However, as with any surgery, there are potential side effects such as temporary or permanent hoarseness or loss of voice, damage to the parathyroid glands, excessive bleeding, formation of a major blood clot in the neck (called a hematoma), and infection (National Cancer Institute [7]).

Radiation therapy is another treatment option and includes the use of radioactive iodine treatment, which is used to kill any remaining thyroid cancer cells after surgery (Mayo Clinic [14]). Chemotherapy may be used in some cases, but it is not as effective for thyroid cancer as it is for other types of cancer. Thyroid hormone therapy is also commonly used to treat thyroid cancer, as it can help to suppress the production of

thyroid-stimulating hormone, which can cause cancer to grow. Targeted drug therapy is a newer treatment option that uses drugs to target specific molecules that play a role in the growth and spread of cancer cells. Finally, watchful waiting may be recommended for patients with small or slow-growing tumors, particularly if the patient is elderly or has other health issues that may make surgery or radiation therapy risky.

Gene

Thyroid cancer commonly involves mutations in the BRAF proto-oncogene and serine/threonine kinase genes, as identified by Xing in 2005. Figure 2 illustrates that these genes encode the protein B-Raf proto-oncogene serine/threonine kinase, which plays a crucial role in the MAP kinase/ERK signaling cascade that regulates cell division, differentiation, and secretion. The gene is located on Chromosome 7,7q34. Mutations in this gene occur in about 44% of papillary thyroid cancer cases, leading to the protein's activation despite signals from other proteins.

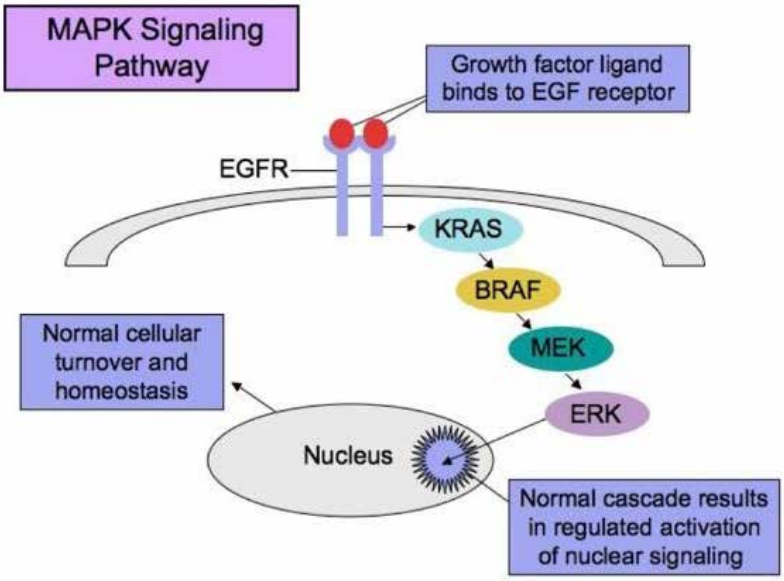

Figure 2. The MAP kinase/ERK signaling pathway. Growth factors bind to their receptors, leading to the activation of the small GTPases H/K and NRAS, which in turn activate the protein kinase B-Raf proto-oncogene serine/threonine kinase (BRAF). B-Raf activates a series of proteins that ultimately activate the extracellular signal-regulated kinase (ERK), leading to the regulation of various cellular processes such as cell division, differentiation, and secretion. Mutations in the BRAF gene, located in chromosome 7q34, are commonly found in papillary thyroid cancer and can lead to constitutive activation of the MAP kinase/ERK pathway (Affiliated Pathologists Medical Group, [20])

SNPs

Single nucleotide polymorphisms (SNPs) are the most common type of genetic variation in humans, marked by differences in a single nucleotide found in more than 1% of the population (Medline Plus, 2020). They occur once every 300 base pairs of sequence on average, with a minor allele frequency (MAF) greater than 1% (Kruglyak and Nickerson, 2001; Stephens *et al.*). As a biological marker, SNPs can be useful in cancer diagnosis, as they can predict an individual's response to specific drugs, susceptibility to environmental factors, and risk of developing cancer.

Methods

To analyze sequence reads, we first downloaded the human reference genome for chromosome 7 from Ensembl. We then selected SRA sequences (accession number PRJNA887246) based on a library strategy and holistic study design of thyroid cancer

and normal patients. The fastq-dump tool was used to download the sequences, which were then checked for quality using the FastQC tool and trimmed as necessary. Poor quality sequences (with a Phred quality score < 33) were identified through the HTML file visualization and trimmed using Trimmomatic. The sequences were then indexed and locally aligned using Bowtie2, and the output SAM file was converted to a BAM file using SAM tools. The BAM file was sorted by coordinates, and read coverage was calculated for each position. To identify single nucleotide polymorphism (SNP) variants, we used the Binary Variant Call Format (BCF) tools.

Results

Our analysis identified various types of genetic variants, including non-coding variants, non-coding transcript exon variants, intergenic SNPs, synonymous variants, missense variants, and regulatory region variants. Non-coding variants are mainly caused by skipping the first or last exon, which could lead to the loss of start or stop codons, respectively (Dhamija & Menon [7]). Non-coding transcript exon variants can involve changes in non-coding exon sequences within non-coding transcripts. Intergenic SNPs can potentially disrupt regulatory elements (Macintyre et al. [11]). Synonymous variants involve codon substitutions that do not alter the encoded amino acid, while missense variants can result in changes to a single base pair, producing a different amino acid than the one normally produced (Edwards et al. [8]). Some missense mutations may impact the function of the encoded protein. Regulatory region variants located in non-coding genomic regions were also detected, which could have a significant impact on the development of diseases (Rojano et al. [14]). These findings suggest a complex and diverse range of genetic variants in our sample, which may contribute to disease susceptibility and pathogenesis (Adeyemo & Rotimi [1]).

Table 1.– Classification of SNPs found. In the 120 individual sequences analyzed, a total of 9 common SNPs were found. This table displays the chromosome position, and SNP accession number, followed by the percentage of individuals containing the particular variation, and then the genetic consequence: intergenic variants (blue), exon variants (turquoise), synonymous variant (green), and not known variants (pink)

Position	Accession Number	Percentage	Consequence
132251222	rs1799238406	98	n/a
149334844	rs928483266	93	exon variant
89638503	rs553702084	88	intergenic variant
109452135	rs1793466465	88	n/a
109452140	rs1254795666	88	intergenic variant
109452143	rs1019099610	88	intergenic variant
109452176	rs1032989423	88	intergenic variant
109452178	rs953608898	88	intergenic variant
55181370	rs1050171	78	synonymous variant

Discussion

Our study identified 9 different single nucleotide polymorphisms (SNPs) and their respective positions, prevalence, and consequences in a population of 102 individuals. We found that at least 78 percent of each SNP was present in the analyzed population, and we listed all the variants for each SNP. Our findings could have implications for detecting thyroid cancer through specific mutations associated with the disease. As DNA sequencing becomes less expensive, individuals may be more likely to undergo early screening for thyroid cancer. To obtain samples for DNA sequencing, thyroid fine needle aspiration biopsy is a minimally invasive procedure that can be used

to remove a small tissue sample from the thyroid gland (Cha & Koo [6]; Johns Hopkins Medicine [19]).

Moving forward, we recommend conducting further research by obtaining thyroid biopsies from a larger and more diverse population, both with and without thyroid cancer, to determine if these same SNPs are present. This research could lead to improved accuracy in detecting thyroid cancer and ultimately better outcomes for patients.

Conclusion

In summary, our analysis revealed significant differences between the thyroid cancer cohort and the normal group, providing evidence that specific single nucleotide polymorphisms (SNPs) are linked to genetic susceptibility to cancer. Our findings support previous research on this topic and contribute to a better understanding of the molecular mechanisms underlying thyroid cancer. These results may have important implications for the development of new screening and treatment strategies for individuals at high risk of developing thyroid cancer. Further research is needed to validate our findings and expand our knowledge of the complex genetic factors involved in cancer susceptibility.

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