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Approach to Follicular Patterned Thyroid Neoplasm and their Molecular Characterization

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Abstract:

Follicular patterned proliferations of the thyroid are considered a challenge for pathologists. Follicular neoplasms of the thyroid gland include benign follicular adenoma (FA), non-invasive follicular tumours with papillary-like nuclear features (NIFTP), and follicular variant of papillary thyroid carcinoma (FVPTC).In Egypt, thyroid tumoursrepresent about 30% of endocrine malignancies. Theyare the sixth most common malignancy among women and the seventeenth among men.Several factors have been involved in the apparent increased incidence of thyroid cancer including the application of more sensitive diagnostic, exposure to radiation, increased iodine intake, chronic lymphocytic thyroiditis, environmental pollutants, and the possible unrecognized carcinogens.

Keywords: Follicular neoplasms, FA, NIFTP, FVPTC

Epidemiology and risk factors

Epidemiology:

Thyroid tumors are the most common neoplasms of the endocrine system; it accounts for 90% of endocrine tumors, and 3.1% of all cancers. (Al-Ibraheem et al., 2023) It is the ninth most common malignancy worldwide (Almansoori et al., 2022)

Thyroid cancer (TC) incidence varies substantially by geographic location, especially in women. In general, the highest incidence is observed in higher-income countries, including the Republic of Korea, Canada, Italy, France, Israel, Croatia, Austria, and the U.S., as well as some middle- to upper-middle-income countries, such as Turkey, Brazil, Costa Rica, and China. (Lortet-Tieulent et al., 2019) Incidence is also high in some island nations and territories, including Cyprus, Cabo Verde, French Polynesia, New Caledonia, and Puerto Rico (Ferlay, et al., 2020)

This variation is thought to be mainly attributable to geographic differences in access to care and diagnostic practices, although environmental exposures also may play a role. (Pizzato et al., 2022)

In the U.S., thyroid cancer is estimated to be the 13th most commonly diagnosed cancer, accounting for nearly 44,000 new cancer diagnoses in 2022 (2.3% of the total), and the 6th most commonly diagnosed cancer among women (Siegel et al., 2022)

The incidence of thyroid cancer is approximately three-fold higher in women than in men (one in 55 U.S. women and one in 149 U.S. men are expected to be diagnosed with thyroid cancer during their life) and increases from adolescence through middle age, peaking around 55 years in women and 65 years in men, and subsequently declining with older age. (Kitahara and Schneider, 2022)

Thyroid cancer mortality is very low relative to incidence (approximately 0.5 deaths per 100,000 per year) with less evidence of a sex disparity. (Ferlay et al., 2020)

In Egypt, thyroid cancer is the sixth most common malignancy among women and the seventeenth among men. (Elbalka et al., 2021)GLOBOCAN 2022 reported that the incidence rate ofdiagnosed thyroid cancer is 2612 cases(1.7% of total cancer cases). it isthe cause of cancer-related mortality in 483 cases representing 0.51 % of total.

Risk factors:

1. Chromosomal (genetic) alterations

In papillary thyroid carcinoma (PTC), activation of the MAPK signaling pathway occurs through two main mechanisms: recombination events and point mutations which are found in almost 70% of papillary cancers

Chromosomal rearrangements such as rearrangement during transfection of proto-oncogene/papillary thyroid cancer (RET/PTC), PAX8/PPARγ, and B-type Raf kinase/A-kinase anchor protein 9 (BRAF/AKAP9) have been associated with exposure to ionizing radiation

Point mutations (RAS and BRAF genes) are most likely result of environmentally-induced or stochastic mutagenesis and are expressed in follicular thyroid cancers. (Bonnefond and Davies, 2014)

PTEN hamartoma tumor syndrome (PTHS) is due to inactivation of PTEN tumor suppressor gene that presents with multiple follicular adenomas with other clinical manifestations. Other genetic mutations in BRAF, NRAS, RET, and KRAS can also cause unexplained follicular adenomas. Follicular adenomas are also part of various syndromes like familial adenomatous polyposis(FAP), Carney Complex syndrome.

Genetic rearrangement of the PAX8-PPAR gene causes loss of follicular growth inhibition, thus facilitating the development of follicular neoplasms. (Carney et al., 2018)

2. Hereditary conditions:

Approximately 3% to 9% of TC is familial non-medullary thyroid carcinoma (FNMTC) with PTC as the most common histologic subtype (85-91%), followed by follicular thyroid carcinoma (FTC) (6-10%), anaplastic thyroid carcinoma (ATC) (1.6%), and Hurthle cell type

carcinoma (HTC). (Peiling and Ngeow, 2011) Familial PTC has more aggressive course, with early metastases, higher recurrence rate, and frequent need for radioactive iodine treatment (McDonald et al., 2011)

Syndromic FNMTC can be found in inherited cancer syndromes as familial adenomatous polyposis, Cowden syndrome, Carney complex and Werner syndrome(Patel et al., 2020)

3. Estrogen

The role of estrogen in development of thyroid cancer is still a matter of debate in numerous epidemiological studies. Some studies showed that exogenous estrogen increases the risk, while early loss of ovarian estrogen lowers the risk for thyroid cancer. Many experimental studies have demonstrated that estradiol behaves as stimulator for either benign or malignant tumors (Luo et al., 2016)

4. Radiation exposure

The most-established risk factor for TC is ionizing radiation especially harmful in children, as thyroid tissue is very radiosensitive at an early age. Ionizing radiation interacts with DNA, causing DNA strand breaks in sensitive sites and somatic mutations, consequently inducing carcinogenesis.It can induce RET/PTC rearrangement (Bonnefond and Davies, 2014)

Medical and dental diagnostics have increased thyroid exposure to radiation, approximately 30% of all CT scans include the head and neck region, radiotherapy for head and neck malignancies,and also dental X-rays could represent risk factor for TC in adult age((Baker and Bhatti, 2006 and Memon et al., 2010)

5. Autoimmunity

The incidence of Hashimoto's thyroiditis (HT) and TC has increased in the past two decades, suggesting a possible correlation. As higher TSH levels in patients with autoimmune thyroid disease stimulate follicular proliferation, follicular epithelial dysplasia in the form of scattered irregular microfollicles without colloid thus promoting the development of PTC. (Khatami 2009) TC could also be induced with production of proinflammatory cytokines and oxidative stress in autoimmune thyroiditis (Vukasović et al., 2012). There are also several similarities between HT and PTC in cytological and immunomarker profiles (Ma et al, 2014).

Patients who have had systemic lupus erythromatosis for many years have found to have a higher risk of developing TC, while hydroxychloroquine therapy could be a protective factor (Guo et al, 2020).

6. Environmental pollutants

Studies investigating cancer risk factors associated with environmental pollutants found that some industrialized food additives, such as nitrates from cured meat and some vegetables,

can compete with iodine uptake, potentially alter thyroid function, and induce thyroid cancer (Pellegriti et al., 2013 and Cherrat et al., 2014). A study by Ward et al. 2010 has shown that above-average nitrate levels in drinking water resources are also associated with an increased risk of thyroid cancer. Polybrominated diphenyl ethers (PBDEs) that are used in building materials, electronics, furnishings, motor vehicles, airplanes, plastics, and textiles may induce abnormal thyroid cell proliferation, favoring a precancerous state (Zhang et al 2008).

7. Lifestyle and diet

Many studies have tried to find a relationship between dietary habits and TC, but the results were inconclusive. A study conducted in 2010-2011 showed negative correlation between diet patterns rich in fruits and vegetables and TC risk, especially among women aged 50 or older (Liang et al 2020). A positive correlation was observed between the consumption of cruciferous plants (i.e. brussel sprouts and cabbage) and the risk of TC. It has also been found that above-average consumption of fruits like oranges and lemons was associated with TC risk. Other fruit and vegetables intake in general showed a significantly decreased risk of TC. (Choi and Kim 2014)

Physical activity has been suggested to influence thyroid cancer risk by improving DNA repair ability, reducing body fat content, lowering resistance to insulin, and altering circulating inflammatory factors (Samani et al., 2007).

Cigarette smoking is suggested to reduce the risk for PTC and probably FTC in 30-40% of subjects (Kitahara et al., 2012). it can lower the levels of TSH and has an anti-estrogenic effect, which can result in a reduced risk of TC (Derwahl and Nicula 2014).

A hospital-based case-control study in Korea showed that female patients with positive family history, higher body mass index (BMI), non-smokers, non-drinkers, and with lower monthly income, had higher risk for TC growth (Myung et al., 2017).

8. Body weight

The prevalence of PTC was associated with high BMI in men, while there was no statistical difference in women. When multiple risk factors were included as (younger age, higher serum TSH and lower fasting blood glucose level, smaller nodule diameter, and multifocality), obese individuals had a significantly higher risk for malignancy and advanced TNM staging compared with patients with normal weight. As most of the obesity was associated with disruptions in insulin metabolism. Insulin-like growth factor1 (IGF-1), which has structural homology to insulin, binds to the IGF-1 receptor and behaves like a potent growth factor that stimulates malignant transformation, tumor progression, and metastasis (Zhao et al., 2019).

Clinical features

Signs and symptoms:

Most thyroid tumors don't cause any signs or symptoms early in the disease. PTC may be discovered incidentally after CT and MRI examination. Small nodules of PTC or microcarcinomas (less than 1 cm in size) are usually of no clinical significance especially in young patients (less than 40 years), since such individuals have a 20-year survival of greater than 98% even with palpable tumors. (Khan et al., 2010)

As thyroid tumor grows, it may cause:

- Painlessness nodules as neoplastic thyroid nodules are usually painless (fig 1)
- Heat intolerance and palpitations suggest an autonomously functioning nodule, which is typically benign (FA).Patients with PTC usually present with a cold nodule on radioactive iodine scan.
- Cervical lymphadenopathy. Involvement of the Delphian lymph node is an adverse prognostic sign in PTC, since this may indicate advanced disease with a need to examine the central and lateral lymph node compartments more carefully. (Khan et al., 2010 and Isaacs et al., 2008)
- New-onset hoarseness of voice: This suggests the involvement of the recurrent laryngeal nerve and vocal fold paralysis
- Dysphagia: May be a sign of impingement of the digestive tract

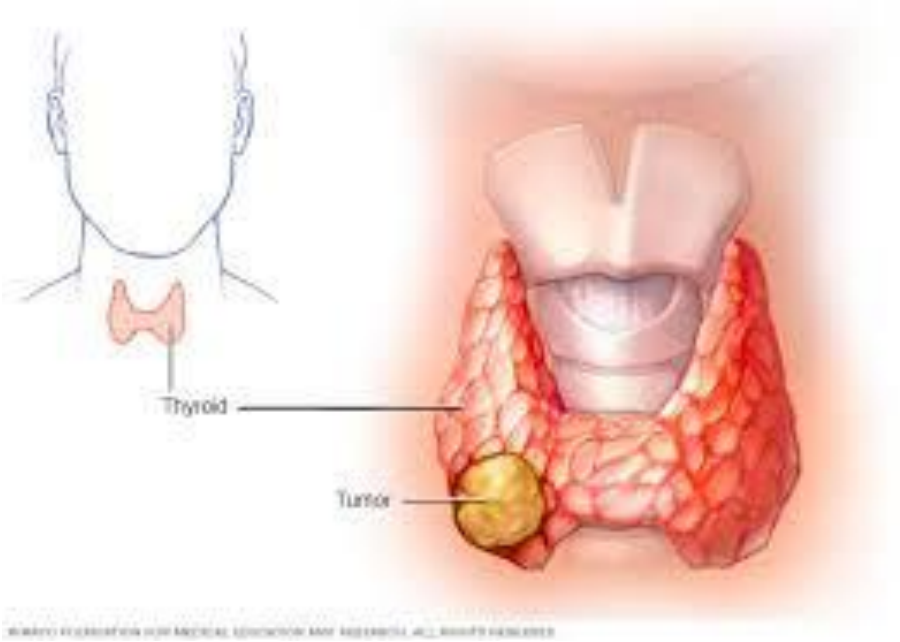


Figure (1): Neoplastic thyroid nodule

WHO classification of thyroid tumors

The thyroid gland gives rise to the most common endocrine tumors, and therefore, it represents the largest chapter in the new 5th edition of the WHO Classification of Endocrine and Neuroendocrine Tumors.

WHO (2017) - Thyroid glandTumors

- Follicular adenoma
- Hyalinizing trabecular tumor
- Other encapsulated follicular patterned thyroid tumors
    1. Follicular tumors of uncertain malignant potential
    2. Well differentiated tumor of uncertain malignant potential
    3. Noninvasive follicular thyroid neoplasm with papillary-like nuclear features
- Papillary thyroid carcinoma
    1. Papillary carcinoma
    2. Follicular variant of PTC
    3. Encapsulated variant of PTC
    4. Papillary microcarcinoma
    5. Columnar cell variant of PTC
    6. Oncocytic variant of PTC
- Follicular thyroid carcinoma (FTC), NOS
    1. FTC, minimally invasive
    2. FTC, encapsulated angioinvasive
    3. FTC, widely invasive
- Hürthle (oncocytic) cell tumors
    1. Hürthle cell adenoma
    2. Hürthle cell carcinoma
- Poorly differentiated thyroid carcinoma
- Anaplastic thyroid carcinoma
- Squamous cell carcinoma
- Medullary thyroid carcinoma
- Mixed medullary and follicular thyroid carcinoma
- Mucoepidermoid carcinoma
- Sclerosing mucoepidermoid carcinoma with eosinophilia
- Mucinous carcinoma
- Ectopic thymoma
- Spindle epithelial tumor with thymus-like differentiation
- Intrathyroid thymic carcinoma
- Paraganglioma and mesenchymal / stromal tumors
    1. Paraganglioma
    2. Peripheral nerve sheath tumors (PNSTs)
        - Schwannoma
        - Malignant PNST
    3. Benign vascular tumors
        - Haemangioma

        - Cavernous haemangioma
        - Lymphangioma
    4. Angiosarcoma
    5. Smooth muscle tumors
        - Leiomyoma
        - Leiomyosarcoma
    6. Solitary fibrous tumor
- Hematolymphoid tumors
    1. Langerhans cell histiocytosis
    2. Rosai-Dorfman disease
    3. Follicular dendritic cell sarcoma
    4. Primary thyroid lymphoma
- Germ cell tumors
    1. Benign teratoma
    2. 9080/1 Immature teratoma
    3. 9080/3 Malignant teratoma
- Secondary tumours

The World Health Organization (WHO) has released the 5th edition of the WHO Classification of Endocrine and Neuroendocrine Tumors, with updates relating to the thyroid gland. The 5th edition divides thyroid tumors into new categories to provide a clearer understanding of their cell of origin, pathologic features, molecular classification, and biological behavior.

The most common thyroid tumors, follicular-cell–derived tumors, are now divided into 3 categories:

- benign
- low-risk
- Malignant neoplasms.

Benign tumors include follicular adenoma and its variants, such as those with papillary architecture.

Non-invasive follicular thyroid neoplasm with papillary-like nuclear features, thyroid tumors of uncertain malignant potential, and hyalinizing trabecular tumor are among the low-risk follicular-cell–derived neoplasms included.

Malignant follicular-cell–derived neoplasms are now stratified on the basis of their molecular profile and aggressiveness. Papillary thyroid carcinomas (PTCs) have many morphological subtypes and are classified as BRAF-like malignancies. In contrast, invasive encapsulated follicular variant PTC and follicular thyroid carcinoma are classified as RAS-like malignancies.

For papillary microcarcinomas, the new classification requires detailed subtyping like that required for tumors greater than 1 cm. This edition also recommends not designating these carcinomas or cribriform-morular thyroid carcinoma as a PTC subtype. In addition, using the term “Hürthle cell” is discouraged.

Furthermore, the 2022 edition does not refer to oncocytic carcinoma as a distinct entity; in the new classification, this term refers to oncocytic follicular-cell–derived neoplasms that lack characteristic nuclear features of PTC and high-grade features. In the new edition, high-grade follicular-cell–derived cancers encompass both poorly differentiated carcinoma and high-grade differentiated thyroid carcinomas.

Squamous cell carcinoma of the thyroid is now classified as a subtype of anaplastic thyroid carcinoma, the most undifferentiated form. The new classification also introduces a grading system for medullary thyroid carcinomas based on mitotic count, tumor necrosis, and Ki67 labeling index. Finally, several unusual neoplasms have been categorized into new sections on the basis of their cytogenesis.

The current classification also emphasized the value of biomarkers that may aid diagnosis and provide prognostic information.(Baloch et al., 2022)

Thyroid neoplasms with follicular architecture can have overlapping morphologic features and pose diagnostic confusion among pathologists. Differentiation between follicular variant papillary thyroid carcinoma and Non-invasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) poses a diagnostic challenge with interobserver variability and is often misdiagnosed as adenomatoid nodule or follicular adenoma.

Follicular adenoma

Follicular adenomas are one subset of benign neoplasms that can occur in the thyroid gland. They typically present as a solitary thyroid nodule or in association with nodular hyperplasia or thyroiditis. (Norris and Farci 2023)

Gross picture:

Follicular adenoma is grossly described as a solitary, encapsulated nodule; the size can be extremely variable, ranging from a few millimeters to 10-15 cm. The color varies from tan to light brown with solid and fleshy appearance. It can resemble multinodular goiter due to secondary changes as hemorrhage and cystic degeneration.

Microscopic picture:

Histological examination of the nodule reveals a follicular architecture present in the entire or nearly entire lesion,the nodule can be described as either microfollicular or macrofollicular growth pattern, and the thyrocytes have a normal cytological appearance. Follicular adenoma is usually a solitary encapsulated lesion and does not have any features suggestive of vascular or/and invasion of the adjoining capsule or to the neighboring thyroid tissue. (Wong et al., 2018 and Baloch and Livolsi 2002)(fig 2)

Variants of follicular adenoma:

- Hyperfunctioning adenoma (Plummer adenoma): formed of thyroid follicles lined by tall columnar epithelium with papillary infoldings, vacuolated cytoplasm, and watery colloid showing scalloping.
- Follicular adenoma with papillary hyperplasia: formed of cystically dilated follicles with intraluminal papillae.
- Lipoadenoma: It considered as an adipocytic metaplasia of the follicular adenoma.

- Follicular adenoma with bizarre nuclei: may be seen after radiation exposure and in hyperfunctioning adenoma.
- Signet ring cell follicular adenoma: follicular adenoma with signet ring cell change.
- Clear cell follicular adenoma: follicular adenoma with clear cell change.
- Spindle cell follicular adenoma: follicular adenoma showed spindle cell metaplasia.
- Black follicular adenoma: it contain black pigment in tumor cell cytoplasm, it occurs in patients on minocycline therapy.(Ricardo, et al., 2017)

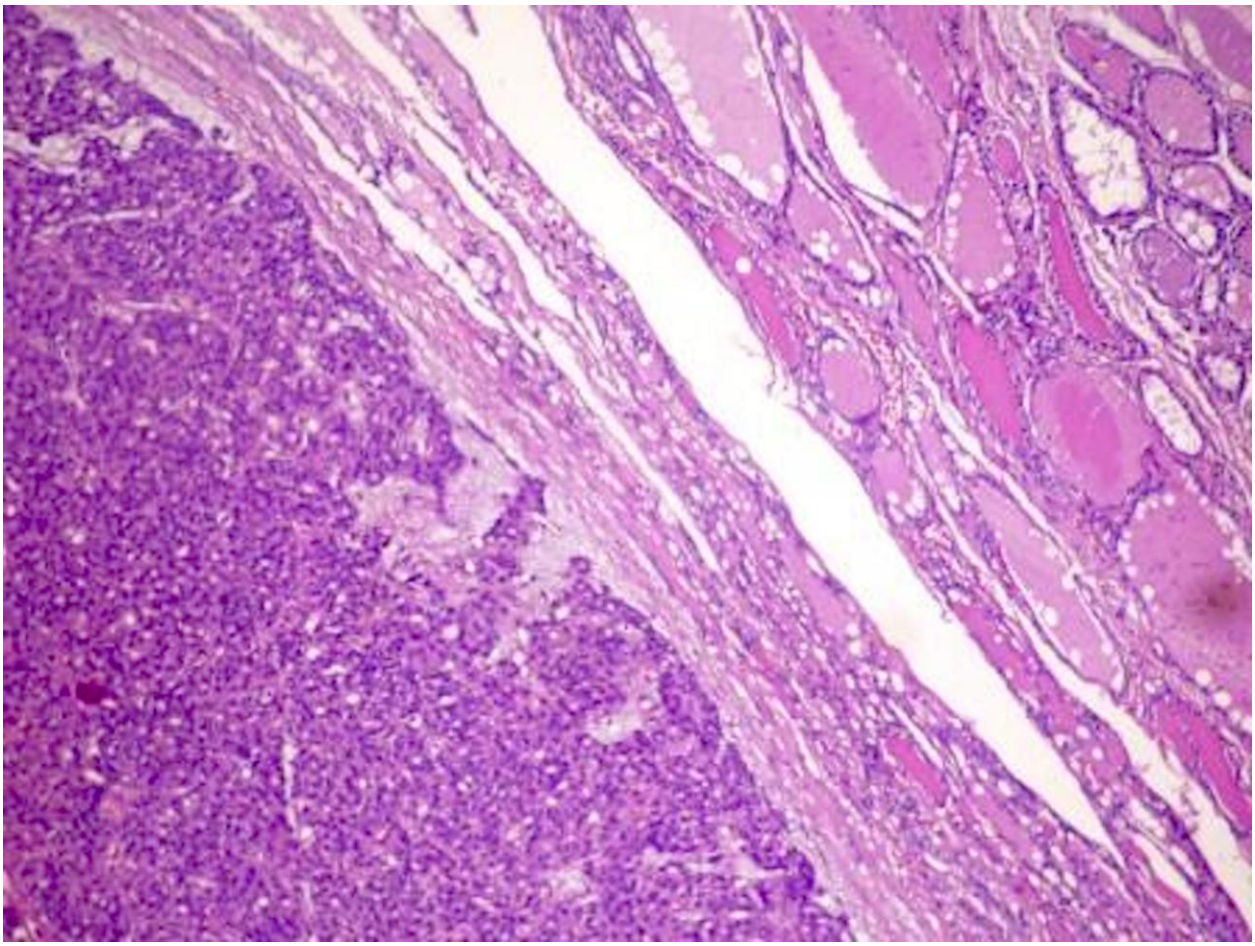


Figure (2): Histopathological picture of FA

Non-invasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP)

In the 1950s and 1960s, thyroid tumor diagnosing was based on the following architecture: papillae were the histopathological mainstay for papillary carcinoma (PTC) and follicles for follicular adenoma/carcinoma. (Tallini et al., 2017)

In 1960, Lindsay recognized, for the first time, the nuclear features of papillary carcinoma in follicular-patterned tumors, but it was later, in 1977, when Chem and Rosai defined the follicular variant of papillary carcinoma (FVPTC).

In 2000, the proposal of "Well-Differentiated Tumor of Uncertain Malignant Potential" (WDT-UMP) characterized as an encapsulated tumor composed of well-differentiated follicular

cells with questionable papillary carcinoma nuclear features and no-invasion was published, but this was not widely accepted. (Tallini et al., 2017 and Williams 2000) The molecular analyses and positive clinical outcome further supported the fact that encapsulated FVPTC is an entity distinct from classical PTC and other variants (Tallini et al., 2017).

Finally, an international multidisciplinary Prof. Yuri Nikiforov led expert panel group including pathologists, clinicians and patients issued a diagnostic consensus on a new entity Noninvasive Follicular Thyroid Neoplasm with Papillary-like Nuclear Features (NIFTP) based on the analysis of 268 cases (Nikiforov et al., 2016)

In 2017, the entity was implemented in a new edition of a WHO classification of tumors of endocrine organs which was also accepted by the American Thyroid Association in their treatment recommendations. (Lloyd et al., 2017 and Haugen et al., 2017).

Microscopic picture

The histopathological diagnostic criteria of NIFTP were revised in 2018. The diagnosis of NIFTP requires the primary criteria defined as encapsulation or clear demarcation of the lesion with no vascular or capsular invasion, follicular growth pattern with no well-formed papillae, no psammoma bodies and less than 30% of solid, trabecular, or insular growth pattern. The assessment of nuclear size and shape, nuclear membrane irregularities and chromatin characteristics concluded as nuclear score of 2 or 3 is required (fig 3). Tumor necrosis and high mitotic activity are not acceptable. The secondary criteria are not required but may be helpful. The secondary criteria include a lack of the BRAFV600E mutation, BRAFV600E-like mutations or other high-risk mutations diagnosed by molecular assays or immunohistochemistry. (Nikiforov et al., 2018 and Alves et al., 2018 and Nikiforov et al., 2016)

The fourth edition of the WHO Classification of Tumors of Endocrine Organs defined NIFTP as follows "non-invasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) which is a non-invasive neoplasm of thyroid follicular cells with a follicular growth pattern and nuclear features of papillary thyroid carcinoma (PTC) that has an extremely low malignant potential (Lloyd et al., 2017). The word "carcinoma" is replaced by the word "neoplasm" as a real-life adoption of a new terminology, rather than as an academic etymology exercise.(Hodak et al., 2016)

Of note, the press reported NIFTP as a benign tumor, despite it was seen as a preinvasive neoplasm best compared to in situ carcinoma of the breast.(LiVolsi and Baloch, 2021) Interestingly, several cases of NIFTP with metastases were recently published. Most of them showed lymph node metastases, though two NIFTP were associated with distant bone and pulmonary metastases as well. (Fakhar et al., 2021, Rosario and Mourao, 2019, Parente et al., 2018 and Cho et al., 2017) If all these NIFTP cases met the revised morphological diagnostic criteria, these findings illustrate very well that despite the prognosis of NIFTP being previously declared as excellent, the risk of adverse outcomes due to metastases is not negligible. Therefore, the continuing follow-up of NIFTP patients is necessary to avoid the unwanted progression of the disease. (Fakhar et al., 2021 and Rosario and Mourao, 2019)

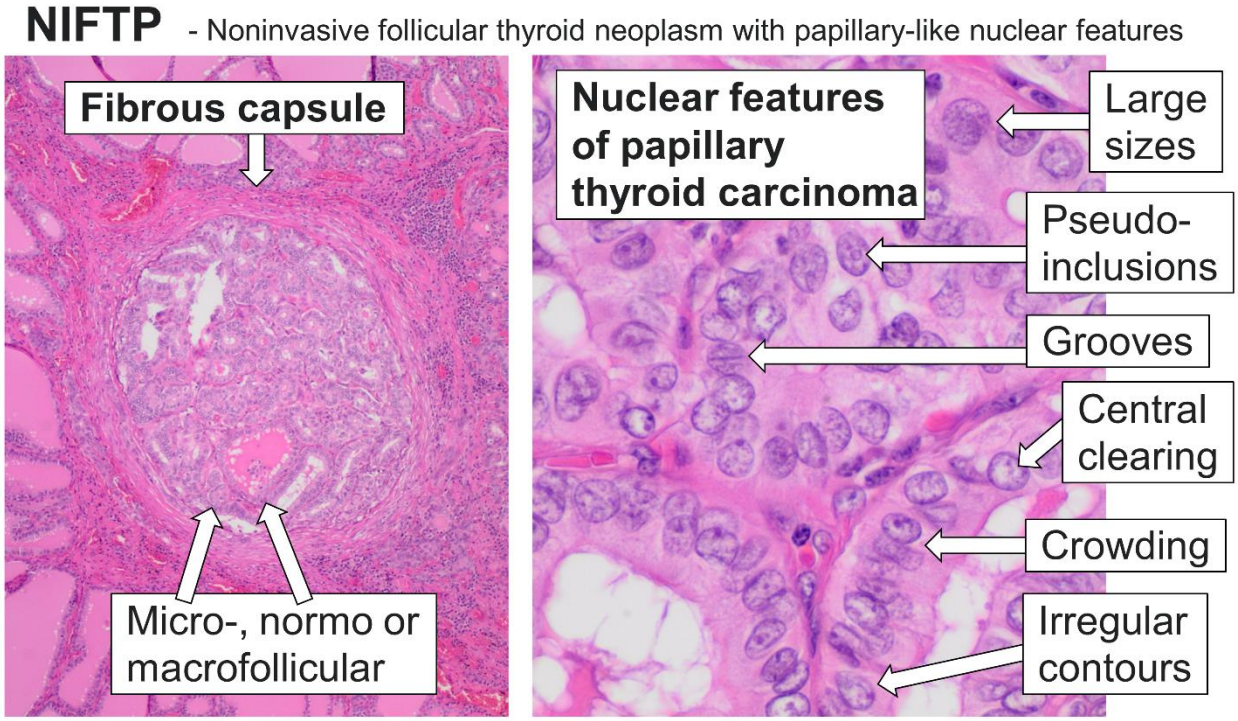


Figure (3): Histopathological picture of NIFTP

Papillary thyroid carcinoma

Papillary thyroid carcinoma constitutes approximately 90% of all malignant thyroid neoplasms. (Kim et al., 2015) Long-term outcomes of patients with papillary thyroid carcinoma are favorable, with a survival rate >90%. (Hundahl et al., 2000 and Do et al., 2017) However, some papillary thyroid carcinoma cases display aggressive oncogenic behavior, and clinicopathological characteristics such as old age, large tumor size, extrathyroidal extension, and cervical lymph node metastasis have been suggested as factors for a poor prognosis. (Ito et al., 2007 and Do et al., 2017)

Papillary thyroid carcinoma development, progression, and metastasis are caused by numerous genetic, reproductive, and environmental factors. (Kim et al., 2015)

Gross picture:

The gross appearance of PTC can be quite variable. Most tumors are firm solid and gray-white, but a significant percentage of tumors may be cystic. It is not uncommon to have a solid primary tumor with a cystic metastasis to a lymph node. PTC may have an infiltrative growth pattern in the thyroid or may show direct extension beyond the thyroid to adjacent tissues. (Khan et al., 2010)

Papillary thyroid microcarcinoma (PTMC) is a specific subgroup of papillary thyroid carcinoma (PTC) and defined by WHO on the largest dimension of 1.0 cm or less. Most of PTMC are not detectable at clinical examination and are diagnosed incidentally during pathologic examination of thyroid specimens after surgery for benign thyroid diseases, or in autopsies. Characteristic cytologic features of PTC help make the diagnosis by FNA or after surgical resection; these include psammoma bodies, cleaved nuclei with an "orphan-Annie" appearance caused by large nucleoli, and the formation of papillary structures. (Dideban et al., 2016)

Microscopic picture:

The conventional PTC shows a papillary architecture with branching. The papillae are covered by cells with eosinophilic cytoplasm and enlarged nuclei. The polarity of the cells may be abnormal or lost in some tumors. Squamous metaplasia may be present. Psamomma bodies with concentric lamellae composed partly of thyroglobulin are more common in some variants of PTC. Some tumors may also contain multinucleated giant cells.(Lloyd et al., 2011)

Cytological features are very helpful in making the diagnosis in some variants of PTC. These consist of enlarged irregular nuclei that are often oval shaped and overlapping because of the nuclear enlargement. The nuclei often show clearing or have a ground glass appearance with prominent nuclear grooves and pink cytoplasmic invaginations.(Limaiem et al., 2024 and Lloyd et al., 2011)

Variant of papillary thyroid carcinoma:

Morphologically, 15 variants of PTC have been identified with various clinicopathologic characteristics and biological behaviors. Some of these variants present prognostic significance, while the other specific subtypes of PTC may behave more aggressively than conventional PTC (Lloyd et al., 2017 and Ricardo et al., 2011)

- Conventional
- Follicular variant
- Papillary microcarcinoma
- Tall cell
- Oncocytic
- Columar cell
- Diffuse sclerosing
- Solid
- Clear cell
- Cribriform morular
- Macrofollicular
- PTC with prominent hobnail features
- PTC with fasciitis-like stroma
- Combined papillary and medullary carcinoma
- PTC with dedifferenatiation to anaplastic carcinoma

Follicular Variant of papillary thyroid carcinoma

The follicular variant of papillary thyroid carcinoma (FVPTC) was first recognized in 1960 and is defined as follicular-patterned thyroid tumor with nuclear features of papillary thyroid carcinoma. (Rosai et al., 1983)

FVPTCs are subdivided into two distinct groups: encapsulated FVPTCs (EFVPTC) and infiltrative FVPTCs (IFVPTC). The prognoses for EFVPTC and IFVPTC have been reported to be quite different; EFVPTC exhibits less lymph node metastasis and less recurrence than IFVPTC, which is similar to classic PTC in terms of invasive growth and lymph node metastasis (Kakudo et al., 2012)

Furthermore, Nikiforov et al., 2016 observed that EFVPTC without capsular or vascular invasion showed an extremely low risk of metastasis to lymph nodes, post-removal recurrence, or other adverse events and thus reclassified it as non-invasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP). This re-classification provided evidence for limited operation (i.e., lobectomy without lymph node dissection) for NIFTP cases. Furthermore, the Japan Thyroid Association recommended active surveillance without diagnostic surgery for indeterminate thyroid nodules including NIFTP. (Bychkov et al., 2018 and Satoh et al., 2017)

Diffuse FVPTC endows a prominent follicular growth pattern without papillary structure, either lacking or rarely showing psammoma bodies, fibrosis, and lymphocytic infiltration. In most cases, it grossly resembles a multinodular goiter instead of a neoplasm with satellite nodules (Ivanova et al., 2002)

These tumors look like follicular neoplasm when examined grossly. They are composed of follicles of variable sizes. The colloid is usually darker or hypereosinophilic compared to the colloid in adjacent non-neoplastic thyroid and may show scalloping "bubble gum" appearance. Occasional multinucleated giant cells are present within the follicles. The cytological features of PTC are important to establish the diagnosis in these tumors (fig 4). The diagnosis of follicular variant of PTC can be quite difficult and controversial. (Wallander et al., 2010)

The prognosis of these tumors is similar to the typical PTC. An exception is the diffuse or multinodular follicular variant, which has a more aggressive clinical course and a high stage at presentation, a notably increased regional lymph node, vascular invasive, and distant metastasis (Vinciguerra et al., 2016).The prognosis of follicular variant of PTC also depends on whether they are completely encapsulated or invasive.(Rivera et al., 2010)

There have been efforts to differentiate NIFTP/EFVPTC preoperatively based on ultrasonogrophic, (Brandler et al., 2018 and Kwon et al., 2018) cytomorphologic**,** (Yang et al., 2017 and Song et al., 2017 and Chandler et al 2017 and Ibrahim et al, 2016) and molecular features (Kim et al., 2018)

Follicular variant of PTC can look like a follicular neoplasm except for the cytological features. Because these tumors can be easily confused with follicular adenomas and follicular carcinomas, the use of immunohistochemical and molecular markers can be very useful in confirming the diagnosis in difficult cases.

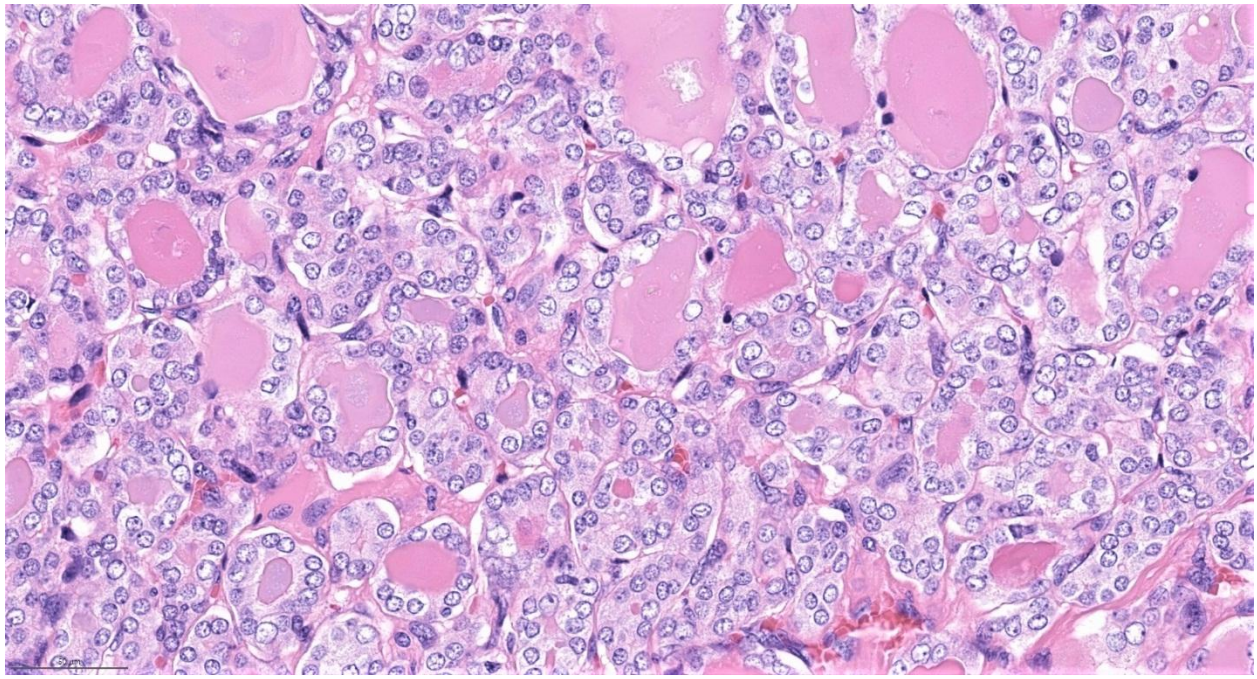


Figure (4): histopathological picture of FVPTC

Staging system of PTC

TNM staging of tumors of the thyroid gland (Filetti et al., 2019)
T- Primary Tumor
TX: Primary tumor cannot be assessed
TO: No evidence of primary tumor
T1: Tumor 2 cm or less in greatest dimension, limited to the thyroid • **Tl a:** Tumor 1 cm or less in greatest dimension, limited to the thyroid • **T1b:** Tumor more than 1 cm but not more than 2 cm in greatest dimension, limited to the thyroid
T2: Tumor more than 2 cm but not more than 4 cm in greatest dimension, limited to the thyroid
T3: Tumor more than 4 cm In greatest dimension, limited to the thyroid or with gross extrathyroidal extension invading only strap muscles (sternohyo1d, sternothyroid, or omohyold muscles) • **T3a**: Tumor more than 4 cm in greatest dimension, limited to the thyroid • **T3b:** Tumor of any size with gross extrathyroidal extension invading only strap muscles (sternohyold, stemothyroid, or omohyoid muscles)
T4: **T4a:** Tumor extends beyond the thyroid capsule and invades any of the following: subcutaneous soft tissues, larynx, trachea, oesophagus, recurrent laryngeal nerve **T4b**: Tumor invades prevertebral fascia or mediastinal vessels, or encases carotid artery
N - Regional Lymph Nodes
NX: Regional lymph nodes cannot be assessed
NO: No regional lymph node metastasis
N1: Regional lymph node metastasis
N1a: Metastasis in Level VI (pretracheal, paratracheal, andprelaryngeal/Delphlan lymph nodes) or upper/superior mediastinum lymph nodes
N1 b: Metastasis in other unilateral, bilateral or contralateral cervical (Levels I, II, Ill, IV, or V) or retropharyngeal lymph nodes
M - Distant Metastasis
MO: No distant metastasis
M 1: Distant metastasis

Stage

1. Papillarythyroid carcinomas; < 55 years
   - Stage I: Any T Any N MO
   - Stage II: Any T Any N M1
2. Papillary thyroid carcinomas>or= 55 years
   - Stage I: T1a,T1b,T2 NO MO
   - Stage II: T3 NO MO or T1 ,T2,T3 N1 MO
   - Stage III: T4a Any N MO
   - Stage IVA: T4b AnyN MO
   - Stage IVS: AnyT Any N M1

Molecular pathology of thyroid tumors

The fourth edition of the WHO Classification of Endocrine Tumors (WHO 4th), published in 2017, included information on the driver genes associated with thyroid tumors. In Follicular Cell-Derived Tumors, genetic abnormalities in the MAP kinase pathway, starting from receptor-type tyrosine kinases such as *RET*, *RAS*, and *BRAF*, are the drivers of carcinogenesis. (Lloyd, et al., 2017 and Nikiforov et al., 2020 and Asa et al., 2022)

Genetic alteration associated with follicular adenoma:

PTEN hamartoma tumor syndrome (PTHS) is due to inactivation of *PTEN* tumor suppressor gene that presents with multiple follicular adenomas with other clinical manifestations. Other genetic mutations in BRAF, NRAS, RET, and KRAS can also cause unexplained follicular adenomas. Follicular adenomas are also part of various syndromes like familial adenomatous polyposis(FAP), Carney Complex syndrome.

Genetic rearrangement of the PAX8-PPAR gene causes loss of follicular growth inhibition, thus facilitating the development of follicular neoplasms. (Carney et al., 2018)

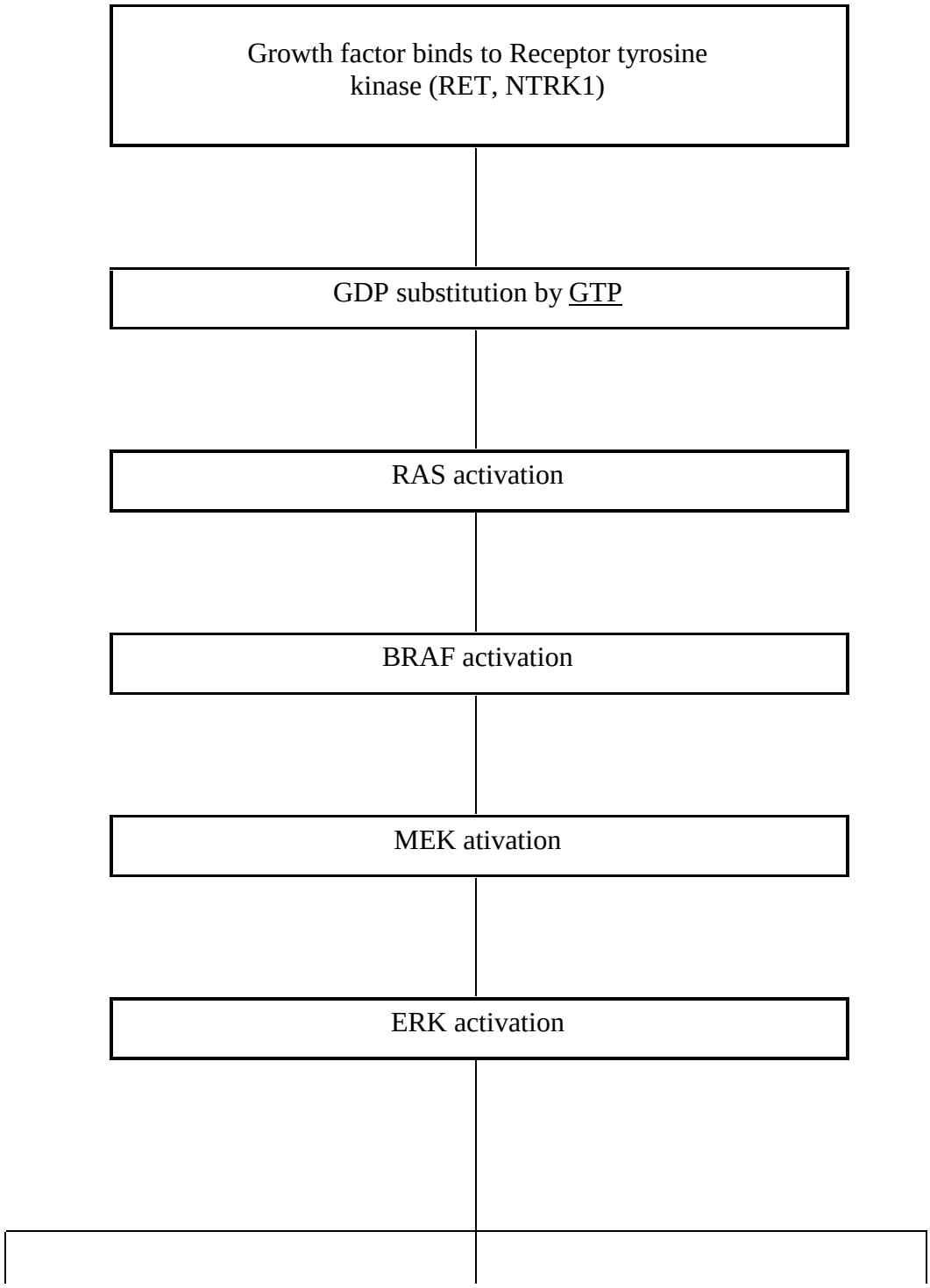
Functioning follicular adenomas occur as a result of a monoclonal expansion of thyroid follicular cells with a high prevalence of activating mutations in the gene for the TSH receptor and less frequently in the adenylate cyclase-stimulating G alpha protein gene that result in increased thyroid hormone secretion independent of TSH. (McHenry and Phitayakorn, 2011)

Genetic alteration associated with papillary thyroid cancer:

- Mutations in RET proto-oncogene
- Mutations in the BRAF oncogene
- RAS mutations
- HMGA2 overexpression

The RET rearrangement encodes for a tyrosine kinase receptor. This rearrangement has also been observed in mice with a history of ionizing radiation exposure. The rearranged form of this gene is well-known as ret/PCT rearrangement and is believed to be related to PTC carcinogenesis.

The mitogen-activated protein kinase (MAPK) pathway is involved in signal transduction of receptor tyrosine kinase such as RET and NTRK1. Tyrosine kinase receptor activation leads to RAS activation which subsequently results in GTP substitution of GDP. The GTP-bound form of RAS makes BRAF active which in turn activates MEK and ERK. ERK is engaged in the regulation of gene transcription including cell differentiation, proliferation, and survival. **(Michael et al., 2010)**


Growth factor binds to Receptor tyrosine kinase (RET, NTRK1)
GDP substitution by GTP
RAS activation
BRAF activation
MEK ativation
ERK activation

cell differentiation	cell proliferation	cell survival

RET proto-oncogene:

The RET gene is expressed in tissues deriving from the neural crest including thyroid C cells and adrenal medulla but it is not expressed in normal thyroid follicular cells (Arighi E. et al., 2005)

The oncogene firstly described was derived from an irradiated PTC. After 3 years, this oncogene was molecularly cloned: it was a chimeric gene generated by the fusion of the RET tyrosine-kinase domain with the 5′ terminal region of a new gene denominated CCD6. This oncogene was named RET/PTC(Wirtschafter A. et al., 1997). 13 different types of RET/PTC rearrangements have been reported and all of them are the result of the fusion of the RET tyrosine-kinase (TK) domain with different genes, that lead to uncontrolled proliferation of the follicular cells harboring the RET/PTC rearrangement and the development of malignancy (Greco A et al., 2009).

RET/PTC1 rearrangements more frequently found in PTC. RET/PTC prevalence in thyroid tumors varies greatly in different series. This difference can be attributed to ethnical and geographic variations as well as to the different sensitivities of detection methods and Tumor heterogeneity. **(**Rao et al., 2014, Al-Humadi et al., 2010, Rhoden K.J et al., 2006 and Zhu Z et al., 2006)

RET/PTC rearrangements are more frequently found in thyroid cancers following radiation exposure (Hieber et al., 2011).In particular RET/PTC3 has been found to be more frequent than RET/PTC1 in post-Chernobyl radiation exposed thyroid cancer especially in those with a short latency period (Williams, 2008)

RET/PTC rearrangements have been also found to be more prevalent in children than in adults both in irradiated and non-irradiated PTC(Su et al., 2016, Elisei et al., 2001)

Other putative carcinogens, like caffeine, ethanol, hypoxia, and others, are able to induce DNA double-strand breaks and generate RET/PTC rearrangements. (Gandhi et al., 2010)

A correlation with a more aggressive phenotype and a more advanced stage has been reported for RET/PTC rearrangements, especially RET/PTC3. In particular, in post-Chernobyl childhood thyroid cancer the RET/PTC3 rearrangement was more frequently associated with the solid variant of PTC which is considered a more aggressive variant and the most prevalent among these irradiated tumors(Prete et al., 2020, Williams, 2008).In a previous study on sporadic PTC they also demonstrated a positive correlation between the presence of RET/PTC3 rearrangement, but not of RET/PTC1, with a bigger size of the tumor and a more advanced stage at diagnosis.(Cordioli et al., 2017, Romei et al., 2008) RET/PTC-positive cases show higher rates of local extension and lymph node metastasis than RET/PTC negative cases. **(**Sergei 2020, Adeniran et al., 2006)

BRAF mutation:

B-Raf is a member of the Raf kinase family of growth signal transduction protein kinases. This protein plays a role in regulating the MAP kinase/ERKs signaling pathway, which affects cell division, differentiation, and secretion. Mutations in the BRAF gene can cause disease in two ways. First, mutations can be inherited and cause birth defects. Second, mutations can appear later in life and cause cancer, as an oncogene.

Inherited mutations in this gene cause cardio-faciocutaneous syndrome, a disease characterized by heart defects, mental retardation and a distinctive facial appearance. (Ou et al., 2024, Roberts et al., 2006)

Acquired mutations in BRAF gene have been found in different tumors, including non-Hodgkin lymphoma, colorectal cancer, malignant melanoma, papillary thyroid carcinoma, non-small-cell lung carcinoma, adenocarcinoma of the lung.BRAF mutation has a crucial role in PTC diagnosis, prognosis, and treatment selection.(Kimbrell et al. 2015)

BRAF mutation in PTC is significantly correlated with large tumor size, positive surgical margins and lymph node metastasis suggesting an association between *BRAF* ^{V600E} mutation and tumor growth and spread. The high frequency of *BRAF*^{V600E} mutation is also found in tall-cell variant, an aggressive variant of PTC, suggests that *BRAF* ^{V600E} mutations might be associated with an aggressive phenotype**.** .(Al-Salam et al., 2020)

RAS oncogene:

Proteins in the RAS family are very important molecular switches for a wide variety of signal pathways that control such processes as cytoskeletal integrity, cellular proliferation, adhesion, apoptosis, and cell migration.

RAS and RAS-related proteins are often deregulated in malignant tumors, leading to increased invasion and metastasis, and decreased apoptosis.RAS activates a number of pathways but the most important one is the mitogen-activated protein (MAP) kinases, which themselves transmit signals downstream to other protein kinases and gene regulatory proteins (Lodish et al., 2000)

In PTC, functional mutation in RAS has been identified in 0–10% of Asian PTC. RAS mutation can promote thyroid tumorigenesis through the *RAS-RAF-MEK-ERK* pathway or through its interaction with PI3K/AKT pathway. (Song et al., 2015)

HMGA2:

This gene encodes a protein that belongs to the non-histone chromosomal high-mobility group (HMG) protein family. HMG proteins function as architectural factors and are essential components of the enhanceosome. This protein contains structural DNA-binding domains and may act as a transcriptional regulating factor.

High expression of HMGA2 is found in a variety of human cancers, but the mechanism by which HMGA2 contributes to the formation of cancer is unknown. (Meyer B et al., 2007) Its

presence is associated with poor prognosis for the patient, but also associated with sensitization of the cancer cells to certain forms of cancer therapy. (Boo et al., 2005)

The oncogenic activity of HMGA includes targeting E2F Transcription Factor 1 (E2F1) and Activator protein 1 (AP1), triggering cyclin A expression, suppression of apoptosis induction by p53, disrupting DNA repair, increasing the expression of inflammatory-related proteins.

HMGA2 overexpression has been also seen in thyroid cancer indicating a prognostic role for HMGA2. (Šamija et al., 2019)Let-7b is a tumor suppressor miRNA whose overexpression in papillary thyroid carcinoma (PTC) is related to inhibition of cancer cell invasion and migration. Indeed, let-7b suppresses the expression level of HMGA2 to exert its function(Li et al., 2017)

In addition, lncRNA ZFAS1 is overexpressed in PTC and exerts tumor promoting function. As it increases HMGA2 expression by acting as a sponge for miRNA-590–3p in promoting tumor cell growth and proliferation (Tong et al., 2019)

The additional genetic alterations may modify the biological behavior of thyroid tumors, for instance TERT reactivation prevents telomere shortening and has been associated with a poor outcome of differentiated thyroid cancer (Song and Park, 2020)