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How does Cyclin D1 behavior as a biomarker in papilliferous thyroid carcinomas and multinodular goiters?

Como a Ciclina D1 se comporta como biomarcador nos carcinomas papilíferos de tireoide e bócios multinodulares?

Ivan José Paredes Bartolomei[1,2], Carmen Australia Paredes Marcondes Ribas[1], Maria Augusta Karas Zella[1], Ana Maria Waaga-Gasser[3], Martin Gasser[1], Nicolau Gregori Czeczko[1], Paulo Afonso Nunes Nassif[1,2], Jose Eduardo Ferreira Manso[4], Orlando Jorge Martins Torres[5]

ABSTRACT

Introduction: Papillary carcinomas are the most prevalent and least aggressive thyroid carcinomas (PTC). In some cases, the diagnosis is doubtfull and the prognosis is poor. The search for tissue biomarkers that ensure both the diagnosis for indeterminate cases and the prognosis, identifying the most aggressive cases, has been studied in recent decades.

Objective: To review the literature in search of cyclin D1 as a marker of papillary thyroid carcinomas and multinodular goiters and evaluate whether its expression correlates with the clinicopathological characteristics of papillary thyroid carcinomas.

Methods: Narrative review carried out by collecting information for reading and analysis from online research on virtual platforms. Initially, a search was carried out from MESH descriptors related to the topic, using the following terms: "papillary thyroid carcinoma, cyclin D1, immunohistochemistry, diagnosis, prognosis." with AND or OR search, considering the title and/or abstract and those chosen were read in full.

Results: The search included 77 articles that were compiled in this review.

Conclusion: Cyclin D1 was expressed in the vast majority of PTCs, with diffuse distribution being predominant. There was no correlation between its expression and any clinicopathological characteristic of PTC.

KEYWORDS: Papillary thyroid carcinomas. Cyclin D1. Immunohistochemistry. Diagnosis. Prognosis.

RESUMO

Racional: Os carcinomas papilíferos são os mais prevalentes e menos agressivos de tireoide (CPT). Em alguns casos, o diagnóstico é duvidoso e o prognóstico ruim. A busca de biomarcadores teciduais que permitam assegurar tanto o diagnóstico para casos indeterminados, quanto o prognóstico, identificando os casos de maior agressividade, têm sido estudadas nas últimas décadas.

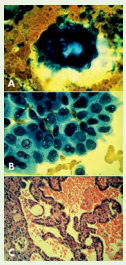
Objetivo: Revisar na literatura na busca da ciclina D1 como marcador dos carcinomas papilíferos de tireoide e nos bócios multinodulares, e avaliar se a expressão dela apresenta correlação com as características clínico-patológicas dos carcinomas papilíferos de tireoide.

Métodos: Revisão narrativa feita colhendo informações para leitura e análise a partir de pesquisa online em platoformas virtuais. Inicialmente foi realizada busca por descritores DECs relacionados ao tema, utilizando os seguintes termos: "carcinoma papilífero de tireoide, ciclina D1, imunoistoquímica, diagnóstico, prognóstico." com busca AND ou OR, considerando o título e/ou resumo e os escolhidos foram lidos na íntegra.

Resultados: A busca incluiu 77 artigos que foram compilados nesta revisão.

Conclusão: A ciclina D1 foi expressa na grande maioria dos CPT sendo a distribuição difusa predominante. Não houve correlação entre a expressão dela com qualquer característica clinicopatológica dos CPT.

DESCRITORES: Carcinomas papilíferos de tireoide. Ciclina D1. Imunoistoquímica. Diagnóstico. Prognóstico.



A) Papillary cancer; B) FNA of a nodule with TLC; C) FNA showing psamoma body

Central Message

Cyclin D1 was expressed in the vast majority of papillary thyroid carcinomas, and the predominant diffuse distribution was used. The benign tissue – multinodular goiter expressed the stain for cyclin D1 with weak intensity and sparse distribution. Cyclin D1 expression did not correlate with clinicopathologic findings (tumor size, angiolymphatic invasion, lymph node metastasis, or distant metastases) in papillary thyroid carcinomas.

Perspective

The analysis of cyclin D1 expression by immunohistochemistry in the differentiation between multinodular goiter and papillary thyroid carcinoma proved useful. This molecular marker could play a differentiating role for clinicians and pathologists in samples acquired by fine-needle aspiration to aid in the diagnosis of papillary thyroid carcinoma in nodules with indeterminate characteristics. However, additional data should be needed to corroborate this hypothesis.

[1] *Faculdade Evangélica Mackenzie do Paraná, Curitiba, PR, Brazil;*
[2] *Mackenzie Evangelical University Hospital, Curitiba, PR, Brazil;*
[3] *Brigham and Women's Hospital, Boston, MA, USA;*
[4] *Department of Surgery, Federal University of Rio de Janeiro, RJ, Brazil;*
[5] *Department of Medicine II, Health Sciences Center, Federal University of Maranhão, São Luis, MA, Brazil.*

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INTRODUCTION

Thyroid cancer is the most common malignant neoplasm of the head and neck region and the most frequent endocrine neoplasia. It predominates in women between 45 and 54 years of age and its main risk factors are female gender, family history of thyroid cancer and exposure to radiation in the neck region. In Brazil, it corresponds to 1.3% of all cancers, with an annual incidence of 9,610 new cases, 4% in women and 0.7% in men.1

The most prevalent types are papillary (80%) and follicular (10%), called differentiated thyroid carcinomas. Medullary and anaplastic carcinomas, on the other hand, are called poorly differentiated and undifferentiated carcinomas, respectively, and are less frequent and more aggressive. Carcinomas may metastasize to the lymph nodes (papillary and medullary), distant metastases (all types), and/or invade local structures such as the recurrent laryngeal nerve, trachea, muscle, and/or esophagus (often in anaplastic lymph nodes).[2]

Its causes and pathophysiology are not well understood.[3,4] Clinically, they manifest predominantly by the appearance of nodules in the thyroid region, less frequently by the appearance of lymph nodes in the cervical chain, hoarseness, dyspnea, dysphagia or cough, and rarely by distant metastases (pulmonary and bone). In recent years, with the advancement of technology, incidental findings by imaging (ultrasound, tomography, resonance imaging or PET-CT) have been increasingly frequent.[5] For diagnosis, in practice, fine-needle aspiration puncture (FNA) is used, which is less invasive and generally effective. However, in certain cases, difficulties may occur in identifying the material as benign or malignant, leading the patient to surgical treatment.

Tumor markers have been used for some decades to minimize the lack of diagnostic and prognostic accuracy in these tumors. For example, in blood, the presence of thyroglobulin correlates with the persistence of differentiated thyroid carcinomas post-thyroidectomy, calcitonin and carcinoembryonic antigen, with the tumor mass of medullary carcinomas. In tumor tissue, the genetic marker BRAFV600E correlates with a worse prognosis in papillaries.[2,6-10]

To aid the interpretation of cytology, molecular analyses through immunohistochemical techniques have also been used in tumor samples. In this direction is cyclin D1, a protein responsible for controlling and coordinating the cell cycle. The dysregulation of this protein can cause its overexpression, affecting the control of the cell cycle, contributing to tumorigenesis.[11,12]

The objectives of this study were to review the immunohistochemical presence of the molecular marker cyclin D1 in papillary thyroid carcinomas and multinodular goiters, and to evaluate whether its expression correlates with the clinicopathologic characteristics of papillary thyroid carcinomas.

METHOD

The narrative review was carried out by collecting information published in indexed journals. The material for reading and analysis was selected from an online search on virtual platforms (SciELO – Scientific Electronic Library Online, Bibliomed, VHL – Virtual Health Library, Pubmed and Scopus). Initially, a search was performed for DECs descriptors related to the theme, using the following terms: "papillary thyroid carcinoma, cyclin D1, immunohistochemistry, diagnosis, prognosis." with AND or OR search, considering the title and/or abstract. With these data, the search included 77 articles that were studied in full by the authors.

DISCUSSION

Thyroid cancer

It often presents as an asymptomatic nodule, discovered by the patient himself (self-palpation) or by the doctor (physical examination). The risk of malignancy of solitary cold nodule or multinodular goiter is 5-8%, being higher at the extremes of age.[13] Less commonly, it presents as a complaint related to the advanced tumor itself, such as hoarseness or paralysis of the vocal cord (due to recurrent laryngeal nerve involvement), tracheal deviation and dyspnea (due to gland enlargement and pressure on the trachea), dysphagia (due to pressure on the esophagus), cervical lymphadenopathy (due to locoregional metastases) or, more rarely (2-5%), due to complaints or discoveries related to distant metastases, especially pulmonary and bone metastases.[13,14]

With the advent of technology, the finding of an incidental nodule is increasingly frequent; however, asymptomatic screening, either by palpation or imaging, is not recommended and may be considered in patients with a history of neck irradiation in childhood or a family of thyroid cancer or multiple endocrine neoplasia.[5,15-17]

The causes of thyroid cancer are not well understood, but some theories are proposed. Genetic mutations (gene BRAFV600E) and imbalances in the cell cycle between proliferative and inhibitory or apoptotic factors for papillary carcinomas; chromosomal translocations for follicular carcinomas and mutations in the proto-oncogene rearranged during transfection – RET for medullary carcinomas.[3,4,18,19]

The pathophysiological mechanisms are also not well understood. Molecular alterations are suggested, some that may favor cell proliferation (oncogenes, growth hormones, apoptotic and cell cycle inhibitory factors) and others that may hinder tumor suppression. It is believed that thyroid cancer may be a continuum of disease, starting from the well-differentiated tumor to the undifferentiated anaplastic tumor, resulting from early and late genetic alterations.[18,19]

The causes and pathophysiology of each type of tumor are summarized in Table 1.

TABLE 1 — Suggested causes and pathophysiology of thyroid carcinomas

Type	Causes	Pathophysiology
Papillary	BRAFV600E mutations and imbalances in the cell cycle	Genetic alterations (early or late) cause molecular imbalances (cell proliferation and inhibition)
Follicular	Chromosomal translocations	
Medullary	RET mutations	

RET=rearranged during transfection; BRAFV600E=RAF kinase B-type gene

Thyroid follicular cells originate more than 90% of cancers (papillary, follicular and anaplastic). The differentiated ones (papillary and follicular) have a slow evolution and good prognosis, with a survival of up to 95% in 10 years. Anaplastic lesions, on the other hand, represent 6% and are called undifferentiated, usually found in the elderly and have a more aggressive character with a rapidly unfavorable evolution.[13,20,21]

Another undifferentiated thyroid tumor, but originating from C or parafollicular cells, is medullary carcinoma and represents 2-3%; it is usually sporadic and more frequently familial (due to genetic predisposition) as part of multiple endocrine neoplasia syndrome. These carcinomas may elevate serum calcitonin.[22] Other tumors, also undifferentiated, are much less frequent and represent ≤1%. In this group are lymphoma, sarcoma, primary non-epithelial tumors and metastatic tumors from other organs to the thyroid such as breast, kidney and melanoma.[20]

In the context of diagnostic tests, the highlight is thyroid Doppler ultrasound, which can be used to guide fine needle aspiration (FNA) at the time of puncture of the suspicious nodule (>1 cm, presence of microcalcifications, length greater than width, hypervascularity, hypoechogenicity, and irregular margins). It is also used to define the dimensions of the nodules and their characteristic aspects (whether solid or cystic), in addition to the evaluation of cervical lymph nodes.[13]

Cytological analysis by FNA usually defines the type. However, in certain situations, histology cannot differentiate carcinomas from follicular adenomas or multinodular goiters and in these cases, the nodule must be removed for anatomopathological analysis through microscopy to observe if there is capsular or vascular invasion. However, even with this analysis, the results may still be indeterminate and should be viewed as suspicious of malignancy.[13]

Ultrasound elastography can be used to assist in the choice of patients who are candidates for surgery when cytology after puncture is indeterminate. The presence of low elasticity indicates surgical treatment.[23,24]

Molecular analyses of indeterminate cytology samples can also be used to detect mutations in BRAFV600E, RET/PTC or RAS, improving the diagnosis. However, some papillary or follicular carcinomas may not mutate. On the other hand, to avoid prophylactic removal of clinically negative lymph nodes in people with thyroid cancer, sentinel lymph node biopsy can be performed using blue dye, radioisotopes, or combined techniques to detect which are truly compromised.[11,13,25-27]

Other diagnostic tests have their specific applications. Core biopsy (lymphoma), neck tomography (medullary carcinoma or lymphoma), serum calcitonin (medullary carcinoma), genetic testing when mutations of the proto-oncogene rearranged during transfection (RET) are suspected in suspected multiple endocrine neoplasia. Laryngoscopy, in patients who have hoarseness due to advanced cancer (they may show paralyzed vocal folds). Hormonal tests (TSH, free T4, and free T3) and I-123 scintigraphy may be considered in the evaluation of functioning (or "hot") nodules when hyperthyroidism is suspected.[28]

The standard treatment for most differentiated tumors (papillary, follicular) is operative followed by radioiodine ablation and suppression of thyroid-stimulating hormone (TSH). Total thyroidectomy is performed if there are any unfavorable prognostic factors (male gender, advanced age, or larger nodule extension/size). Complications may occur in 2% of cases (recurrent laryngeal nerve injury and/or hypoparathyroidism). Hemithyroidectomy (lobectomy associated with isthmectomy) is performed if the carcinoma is unilateral, smaller than 1 cm and if there are no unfavorable prognostic factors.[13]

Serum thyroglobulin should not be used as a diagnostic tool, but it is useful in the post-treatment monitoring of differentiated carcinomas. Low levels observed before radioiodine ablation can predict the future disease-free state.[29]

If the disease relapses or metastasizes, I-131 radioiodine ablation follows with or without reoperation depending on accessibility to the tumor. If it does not respond to radioiodine, systemic treatments with kinase inhibitors (sorafenib and lenvatinib) are used, but with significant toxicities.[30-32]

Medullary carcinomas should be treated with total thyroidectomy and in cases of recurrence there will be a need for additional surgery, associated with radiotherapy. For advanced cases or those who are not candidates for surgery, the oral tyrosine kinase inhibitor (vandetanib) proved effective compared to placebo.[33,34]

Anaplastic carcinomas should be treated, if possible, with total thyroidectomy followed by chemotherapy (adriamycin or platinum) and radiotherapy; primary thyroid lymphoma with radiotherapy and chemotherapy (cyclophosphamide, doxorubicin, vincristine, and prednisolone or regimen.[35]

Table 2 summarizes the characteristics of thyroid tumors, as well as their treatments and survival rates.

Papillary thyroid carcinomas (TLC)

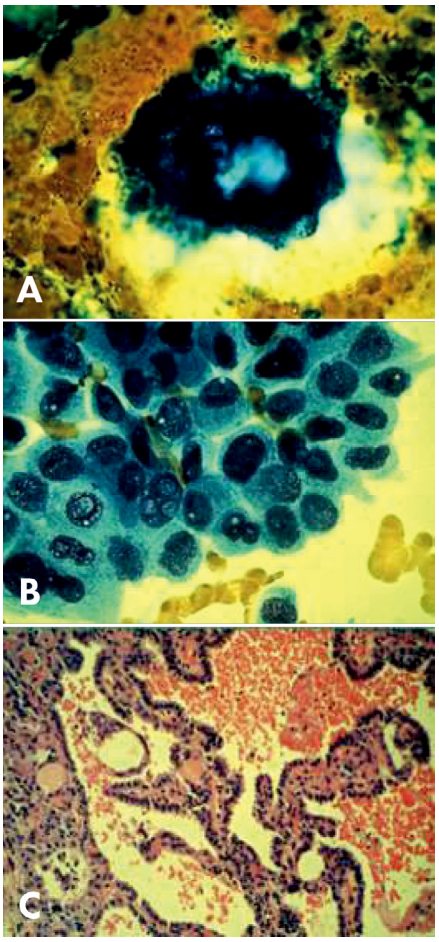
Histologically, they are non-encapsulated and partially cystic tumors, characterized by the presence of papillae (one to two layers of tumor cells that surround a well-defined fibrovascular center) without follicles or colloids (Figure 1A). Morphological

TABLE 2 — Main characteristics of the types of thyroid cancer

Cels. Origin	Type	Differentiation	Features	Matastasis	Blood marker	Treatment	Survival
Follicular	Papillary (80%)	Differentiated	Multifocal or multicenter (25%)	Ganglia (60%)	Thyroglobulin	Cx, I131, LT4	> 90%(10 years)
	Follicular (10%)	Differentiated	Unifocal	Lung and Bone (20%)	Thyroglobulin	Cx, I131, LT4	80%(10 years)
	Anaplastic(6%)	Undifferentiated	Rapid progression, Local invasion (recurrent laryngeal, trachea, muscle and/oresophagus)	Blood	-	Cx QT, RT	Months
Parafollicular	Medullary (2%)	Little Differentiated	Multricentric.Family forms (25%)	Ganglia and Blood	Calcitonin CEA	Cx, RT ITQ	80%(5 years)

Cx=surgery; RT=radiotherapy; QT=chemotherapy; LT4=levothyroxine; ITQ=tyrosine kinase inhibitor; I131=radioiodine 131

diagnosis is based on typical cytological findings, but none of them are pathognomonic in isolation, such as large, oval, large and overlapping nuclei, which may contain hypodense chromatin and cytoplasm with pseudoinclusions due to the redundant nuclear membrane or nuclear sulci (Figure 1B). Approximately half of papillary thyroid cancers contain calcified psamoma bodies, or remnants of infarcted tumor papillae (Figure 1C).[14]



Source: Tuttle, 201814

FIGURE 1— Papillary thyroid carcinoma: A) surgical specimen showing the classic histological appearance of papillary cancer with papillary structure and without follicles or colloids, and follicular development can be observed in some of these carcinomas (follicular variant of papillary cancer) and in them, the diagnosis is made based on the cytological characteristics of the cells; B) FNA of a nodule with TLC where the cells and nuclei are large and the cytoplasm has a "ground-glass" appearance and the nucleoli are prominent and nuclei with slits, grooves and "holes" due to intranuclear cytoplasmic inclusions ("Annie's orphan eyes"); C) FNA of a nodule with TLC where aspirated with a fine needle showing psamoma body.

There are variant forms of TLC that have all the histological characteristics with some variations, such as the follicular variant (more frequent and less aggressive) and the high cell, insular, columnar, hobnail, oxyphilic or Hürthle cell, trabecular, clear cell, cribriform and diffuse sclerosing variants (less frequent and more aggressive).[36]

The most common variant of papillary carcinoma is follicular and has small follicles similar to those seen in follicular carcinomas. There are 2 subtypes, the non-invasive (encapsulated and well circumscribed) and the other invasive (capsule or vessel or without a well-defined capsule). Because the prognosis of noninvasive follicular variant of papillary thyroid carcinoma (NIFVPTC) is excellent, the American Thyroid Society (ATA) removed the term carcinoma, proposing the term noninvasive follicular thyroid neoplasm with Papillary-like Nuclear Features (NIFPT), which is managed as a neoplasm rather than malignancy and treated only with lobectomy, without ablation or suppressive therapy of TSH.[37,38]

The growth pattern and biological behavior of papillary cancer is variable. At one end of the spectrum is common papillary microcarcinoma, formerly called occult papillary cancer, defined as a tumor less than 1 cm in diameter. These microcarcinomas are found in 15-30% of the thyroid glands at autopsy. The high frequency, associated with the rarity of clinically detected papillary cancer, suggests that the presence of a single focus of microcarcinoma in thyroidectomy is probably an incidental finding and of no clinical importance. At the other end of the spectrum is locally invasive cancer, with distant metastases seen at the time of diagnosis. These tumors are prone to metastasize through the intrathyroid lymphatic channels and form multifocal tumors or involve regional lymph nodes.

Metastases to lymph nodes are the most frequent and occur in up to 80% of cases (in microcarcinomas the incidence can reach 64%). There may be capsular or vascular invasion of the lymph node or, less frequently, of the blood vessel, resulting in distant metastases (2-10%), most of which are to the lung and less frequently to the bones. Rarer sites are the brain, kidneys, liver, and the adrenal glands.[14]

The mortality of papillary carcinomas is determined by age, tumor size, locoregional invasion, and distant metastases. Mortality with papillary cancer without metastases is only 6%.[16]

Molecular characteristics have been used as predictors of disease extent. Thus, the presence of BRAFV600E mutations, telomerase reverse transcriptase (TERT) and expression of vascular

endothelial growth factor (VEGF) correlate with the extent of the disease and may be useful in risk stratification.[4,39]

In addition, other factors associated with increased risk of both recurrence and death are: intrathyroid multicentricicity, bilateral lymph node or mediastinal involvement, more than 10 lymph node metastases, lymph node metastases with extranodal extension, male gender, and delay in surgical resection after 1 year of nodule diagnosis.[14,21]

Finally, postoperative staging is important for the evaluation of prognosis through clinical-histological crossovers to predict both survival and tumor recurrence. The most used classifications are Tumor Node Metastasis – TNM (risk of mortality) and American Thyroid Association – ATA (risk of recurrence).[40,41]

In the AJCC/TNM 2017 staging (Table 3), the 10-year survival at the cut-off age of 55, in stages I to IV are 99.5%, 94.7%, 94.1%, and 67.6%, respectively, providing the prognostic estimate for those who remain at high risk.[42] The use of the modified initial risk stratification system of the ATA (Table 4) helps to assess the risk of disease recurrence in patients after initial therapy

TABLE 3 — TNM AJCC UICC Staging 2017. DTC: in TNM, all categories can be subdivided: solitary tumor(s) and multifocal tumor(m), with the largest tumor determining the classification

Primary tumor (T)				
Category	Criterion			
TX	Primary tumor cannot be accessed			
T0	No evidence of primary tumor			
T1	Tumor ≤ 2 cm limited to the thyroid			
Tier 1a	Tumor ≤ 1 cm limited to the thyroid			
T1b	Tumor > 1 cm but ≤ 2 cm limited to the thyroid			
T2	Tumor > 2 cm but ≤ 4 cm limited to the thyroid			
T3	Tumor > 4 cm limited to the thyroid or extrathyroid extension Gross invading only muscle			
S3a	Tumor > 4 cm limited to the thyroid			
T3b	Coarse extrathyroid extension that invades only the muscles (sternohyoid, sternothyroid, thyrohyoid or omohyoid muscles) of a tumor of any size			
T4	Includes coarse extrathyroid extension			
Tier 4a	Coarse extrathyroid extension invading subcutaneous soft tissues, larynx, trachea, esophagus, or recurrent laryngeal nerve of a Any size			
T4b	Coarse extrathyroid extension invading the prevertebral fascia or involving the carotid artery or mediastinal vessels of a Any size			
Regional lymph nodes (N)				
Category	Criterion			
NX	Lymph nodes cannot be accessed			
N0	No evidence of locoregional lymph node metastases			
N0a	One or more lymph nodes confirmed to be benign in pathology			
N0b	No clinical or radiological evidence of lymph node metastases Local-regional			
N1	Metastases to regional lymph nodes			
N1a	Metastases to level VI or VII lymph nodes (pretracheal, paratracheal or paralaryngeal or mediastinal This can be unilateral or bilateral			
N1b	Metastasis to unilateral, bilateral, or bilateral lateral cervical lymph nodes contralateral (levels I, II, III, IV, or V) or retropharyngeal lymph nodes			
Distant metastases (M)				
Category	Criterion			
M0	No distant metastases			
M1	Metastases at a distance			
Staging				
If age of diagnosis is...	If T is ...	If N is ...	If M is ...	Therefore, the internship is
< 55 years old	Any T	Any N	M0	I
< 55 years old	Any T	Any N	M1	II
≥ 55 years old	T1	N0/NX	M0	I
≥ 55 years old	T1	N1	M0	II
≥ 55 years old	T2	N0/NX	M0	I
≥ 55 years old	T2	N1	M0	II
≥ 55 years old	T3a / T3b	Any N	M0	II
≥ 55 years old	Tier 4a	Any N	M0	III
≥ 55 years old	T4b	Any N	M0	VAT
≥ 55 years old	Any T	Any N	M1	Ivb

AJCC=American Joint Committee on Cancer

Source: Table modified by the author from AJCC Cancer Staging Manual, Eighth Edition (2017)

TABLE 4 — Risk stratification of persistence or recurrence (ATA 2015)

Low	Intermediary	High
Papillary thyroid carcinoma with all features present	Any of the following gifts	Any of the following gifts
No locoregional or distant metastases	Microscopic invasion of peratireoid soft tissues	Invasion of a macroscopic tumor
The entire macroscopic tumor was resected	Metastases in cervical lymph nodes or I131-avid metastatic foci in the neck on post-treatment examination performed after thyroid ablation	Incomplete tumor resection with gross residual disease
No loco-regional encroachment	Tumor with aggressive histology (high cell, insular, columnar, Hürthle, follicular carcinoma, Hobnail) or vascular invasion	Metastases at a distance
No aggressive variant histology (high cell, insular, columnar, Hurthle, follicular carcinoma, hobnail)	Clinical N1 or > 5 Pathologic N1 with all lymph nodes involved <3 cm in the largest dimension*	Postoperative serum thyroglobulin suggestive of distant metastases
No vascular invasion	Multifocal papillary thyroid microcarcinoma with extrathyroid extension and BRAFV600E mutation (if known)*	Pathologic N1 with any metastatic lymph node ≥ 3 cm in its greatest dimension*
No uptake of I131 outside the thyroid		Follicular thyroid cancer with extensive vascular invasion (> 4 foci of vascular invasion)*
Clinical N0 or pathological N1 (<0.2cm in the largest diameter)* with ≤ 5 micrometastases		
Intrathyroid follicular variant papillary thyroid carcinoma, encapsulated*		
Well-differentiated intrathyroid follicular thyroid carcinoma with minimal or no capsular invasion vascular invasion (<4 foci) *		
Intrathyroid, unifocal, or multifocal papillary microcarcinoma, including the mutation BRAFV600E if Known*		

Source: Table modified by the author from Haugen BR et al., 201621

ATA=American Thyroid Association, 2015; I131=Iodine 131; BRAFV600E=RAF kinase gene type B; *=proposed modifications, not present in the initial risk stratification system in 2009.

Biomarkers

They are substances produced by the body's cells in response to the physiological or pathological state, such as in cancer. They can be present in various body fluids and tissues such as blood, urine, feces, and in the tumor tissues themselves.[43]

In oncology, biomarkers are called tumor markers and have several clinical applicabilities related to the approach to the disease (risk, screening, diagnosis,

prognosis, response to treatment, monitoring, and recurrence). However, there are many limitations to its use in clinical practice, as several clinically benign situations can also cause an increase in its concentrations, and not all cancer patients have an increase in the marker associated with neoplasia. Therefore, the measurement of the markers should be combined with clinical findings and biopsies to optimize their interpretation.[43]

Biomarkers can be metabolites, carbohydrates, peptides, proteins, platelets, T lymphocytes, autoantibodies, and genes. Historically, the first biomarker used was Bence Jones' protein, researched in the urine of a patient with multiple myeloma in 1847. Since then, other biomarkers have been used for cancer, such as carcinoembryonic (colon) antigen, alpha fetoprotein (liver), prostate-specific antigen (prostate), human chorionic gonadotropin (testis), and, more recently, genetic biomarkers such as mutations in the BRCA1 and BRCA2 genes (breast and ovarian cancer).[43]

The discovery of new biomarkers is closely linked to the evolution of molecular technologies. Thus, new protein expressions and new genetic markers have aroused interest in the early detection of cancer, in the evaluation of tumor aggressiveness, and in the ability to predict recurrence.[43,44]

Biomarkers in papillary thyroid carcinomas

When fine-needle aspiration puncture (FNA) of a suspicious nodule in the thyroid gland is performed, there are sometimes doubts in the morphological identification between malignant and benign lesions (multinodular goiter and thyroiditis).[45] The use of immunohistochemical markers in these cases helps in the definition of the diagnosis and in the stratification of risk.[46] Table 5 lists the main genetic tissue immunomarkers used for papillary thyroid carcinomas and their variants.

TABLE 5 — Morphological and molecular parameters of TLC

Type of tumor	Morphology	Molecular Marker
Classic	Clear papillae and nuclei	BRAFV600E, RET/PTC fus,NTRK fus, ALK fuses, 1q amp
Follicular variant	Clear follicles and nuclei	BRAFV601E, RAS, PAX8/PPARγ EIF1AX, THADA fus, 22q del
Other variants: tall cells, columnar, solid, hobnail	Structures and features special cell phones	BRAFV60E, 1q amp, TERTpromoter, TP53, PIK3CA, CTNNB1

Source: Adapted from Filetti et al. (2019).46. 22d del=deletion of 22q; amp=amplification; ALK=anaplastic lymphoma kinase; CNA=copy number alteration; CPT=papillary thyroid carcinomas (PTC); del=deletion; fus=merge; CTNNB1=catenin beta 1; EIF1AX=eukaryotic translation initiation factor 1A X-linked; HBME1=Hector Battifora mesothelial–1; NTRK=tropomyosone kinase receptor or neurotrophic tyrosine kinase receptor type; PPARγ=peroxisome proliferator activated receptor gamma; PIK3CA=phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; PAX8=paired box gene 8; WHO=World Health Organization; RET=rearranged during transfection; TERTpromoter=telomerase promoter reverse transcriptase; TP53=tumor protein p53.

In recent years, immunohistochemical markers have been used to differentiate malignant and benign thyroid lesions. In this direction, HBME1 (Hector Battifora mesothelial–1), galectin-3 and CK19 (cytokeratin 19), CD56, CD57, CD44v6, Rb-1, p53, E-cadherin and bcl-2, CITED-1 are used, used to differentiate classic papillary carcinomas from other lesions in combination panels.[47-49]

Traditional proliferative immunohistochemical markers such as Ki-67, p27/kip1, and cyclins D1 and E, were evaluated in the differential diagnosis of differentiated carcinomas vs. benign tissues, and then as prognostic parameters in the same neoplastic category with controversial results.[50-52]

Fundamentals of cellular and molecular biology

The cell cycle

The only way to form a new cell is to duplicate the existing one. The set of steps and phases for the cell to be able to duplicate the genetic material contained in its chromosomes and thus be able to create another cell, is called the cell cycle. For this to occur in eukaryotic cells (with cell membrane, nucleus and organelles) 2 phases are necessary: interphase and mitosis.[53] The cell cycle and its different stages are illustrated in Figure 1.

Interphase

It is the preparation phase for cell division. To do so, the cell nourishes itself, grows and duplicates its DNA, so it is the most time-consuming phase and 3 steps are needed to complete it.

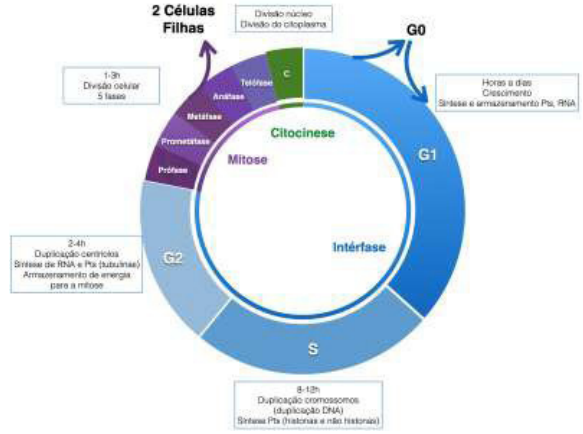
The first is known as phase G1 or interval 1 (gap) and occurs shortly after the end of mitosis and can take hours to days to finish. In this phase, the daughter cells grow (through the synthesis and storage of proteins and RNA) and recover their volume, being able to proceed to the next phase of the cycle (phase S). Cells that are not fit go to rest (G0 phase) and can remain days, months or years before re-entering the cell cycle or even being permanently out of the cycle.

The second, or S phase (synthesis), lasts 8-12 hours, is marked by the synthesis of proteins (histones and non-histones) and duplication of genetic material or DNA (chromosomes and centrosomes).

The third, phase G2 or interval 2, is the one that precedes mitosis and, therefore, is the preparation phase for cell division. It lasts 2-4 h and is marked by the synthesis of proteins (tubulins), RNA, and duplication of centrioles; In this way, energy storage occurs so that the cell can progress to mitosis.[54]

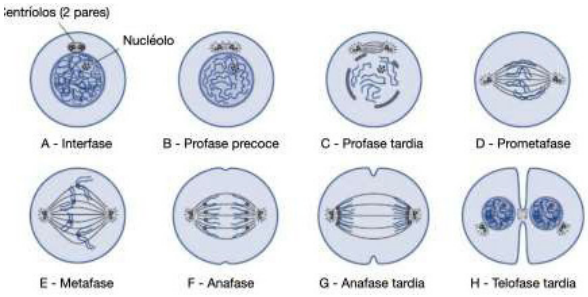
Mitosis

Mitosis or M phase is that of cell division. It lasts 1-3 h, occurs after the G2 phase and completes the cell cycle. There are 5 stages: prophase, prometaphase, metaphase, anaphase and telophase, followed by mitosis (division of the nucleus) and cytokinesis (division of the cytoplasm), individualizing the daughter cells.[54] The different phases of mitosis with their respective characteristics are illustrated in Figures 2 and 3 and summarized in Table 6.



G1 = gap G1; S=synthesis; G2=gap G2; M=mitosis

FIGURE 2 — Phases and stages of the cell cycle: interphase (G1, S and G2); mitosis (prophase, prometaphase, metaphase, anaphase, telophase) and karyokinesis and cytokinesis.



Source: Adapted by the author from Kelley, Wood, Enders (1984).

FIGURE 3 — Phases of mitosis

TABLE 6 — Phases of mitosis

Phase	DNA content	Identified characteristics
Prophase (early)	The DNA content is twice that of the S phase of interphase. Doubling of centrioles	The nuclear envelope and nucleolus begin to disappear. Chromosomes condense; are made up of two sister chromatins joined by the centromere.
Prophase (late)		The centrioles migrate to the opposite poles and give rise to the spindle fibers and of the aster.
Prometaphase	Double DNA rotation	The nuclear envelope disappears. The kinetochores develop in centromeres and forms kinetochoric microtubules.
Metaphase	Double DNA rotation	Maximum condensation of chromosomes, which align in the plane Equatorial of the mitotic spindle.
Anaphase	Double DNA rotation	The daughter chromatins are separated by the centromere.
Anaphase (late)		Each chromatin migrates to the opposite pole of the cell along the microtubule (cariokinesis). A groove of division begins to form.
Telophase	Each daughter cell contains 1 endowment of DNA	The groove (middle body) between the two newly formed daughter cells lies deeper (cytokinesis). The nuclear envelope and nucleoli reappear; The chromosomes disperse and originate a new interphase nucleus.

Source: Adapted by the author from Gartner and Hiatt, 2015.54

Cell cycle regulation and cyclins

In order for the cell cycle to occur harmoniously in all its phases, the cell has a series of controls or checkpoints that initiate, induce and modulate the progression of the cell cycle. Cyclins play a fundamental role in this regulation. They are proteins that regulate cell cycle progression. There are 11 types in mammalian cells (A, B1, B2, C, D1, D2, D3, E, F, G, and H), each expressing themselves more or less intensely depending on the phase of the cell cycle as shown in Figure 4.

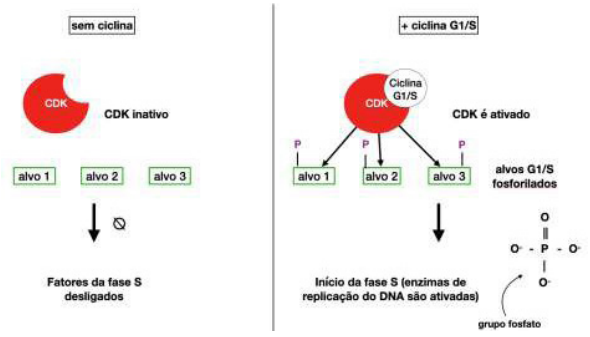
Source: Modified by the author of "Control of the cell cycle" from Open Stax College, Biology. G1 = gap G1; S=synthesis; G2=gap G2; M=mitosis.

FIGURE 4 — Expression of cyclins in the cell cycle: several types of cyclins act in each phase; the predominant cyclins of the G1 phase (cyclin G1 in the figure) are cyclins D and E; cellular progression from phases S to G2 and then to M (cyclin S and M) are coordinated by cyclins A and B; all of these cyclins act in association with their respective cyclin-dependent kinases (CDKs).

Cyclin D1, encoded by the CCND1 gene, is one of the most important cyclins in the cell cycle and predominates in the G1 phase. It is responsible for the control and coordination of the cell cycle.[55,56]

Cyclin-dependent kinases (CDK)

In parallel, the family of enzymes known as cyclin-dependent kinases or CDKs are inactive in cells, but when they bind to cyclins and form the cyclin-CDK complex, they become active by phosphorylating (adding 1 phosphate group PO4) specific target proteins within the cell to trigger responses that stimulate genes involved in DNA synthesis (via retinoblastoma gene phosphorylation – pRB, which releases transcription factors such as E2F-1) stimulating the cell to progress through the cycle (Figure 5).[57,58]



CDK=cyclin-dependent kinases; P=phosphate; DNA: deoxyribonucleic acid

FIGURE 5 — Cyclin-dependent kinases

Cell cycle checkpoints

In the G1 phase, for example, cyclin-dependent kinases (CDK) bind to regulatory cyclins D and E, eliciting responses that allow the cell to advance to the next phase (phase S). A checkpoint between these phases (checkpoint G1/S or R point) ensures supervision, preventing the cell from progressing to the next phase with flaws in DNA duplication, such as inadequate number of organelles or cell growth. Cellular progression from the S to G2 and

then to M phases are coordinated by the cyclin A-CDK and cyclin B-CDK complexes respectively. Before the cell enters mitosis, there are 2 control points that ensure the quality of the DNA, the G2/M point (unduplicated DNA control point) and the DNA/G2 (DNA damage control point). Finally, in the mitosis phase (phase M), the spindle assembly checkpoint (at the beginning of mitosis) and another checkpoint of the identical chromosome distribution or M/G1 point (at the end of mitosis) qualify the cell to finish the cycle (Figure 5).

Cyclin-dependent kinase inhibitors (CDKi)

While there are cyclin-dependent kinases (CDKs) that stimulate cell cycle progression after being bound to their respective cyclins, on the other hand, inhibitors of cyclin-dependent kinase or CDKi (inhibitor of cyclin-dependent kinase) proteins inhibit cycle progression. Examples of these inhibitors are the proteins p15, p16, p18 and p19 (inhibit CDK4 and CDK6 and block the cell cycle at the restriction point R) and the proteins p21, p27 and p57 (block the cycle at all set points (Figure 6).[59]

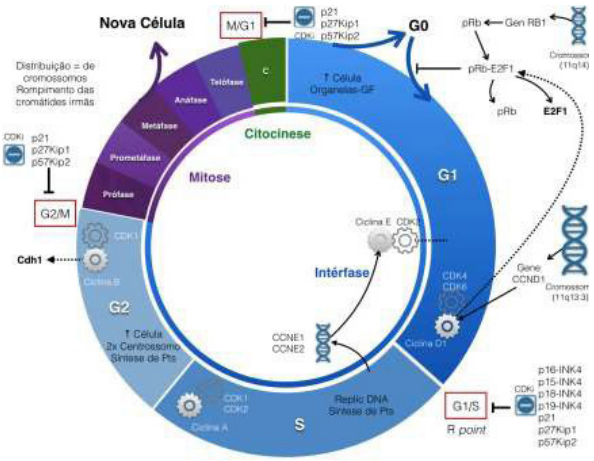
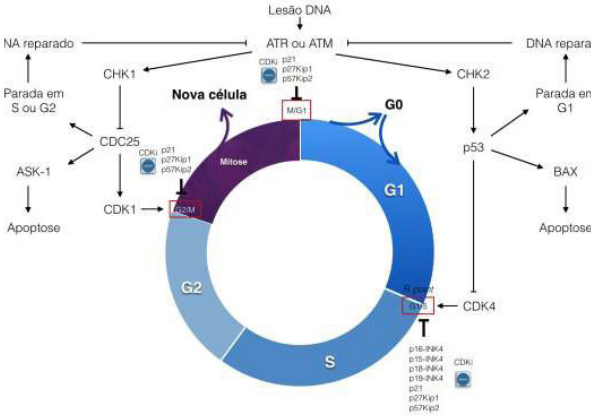


FIGURE 6 — Cell cycle regulation system: Gen RB1 (retinoblastoma gene, was the first tumor suppressor gene described - 1986) encodes protein (pRb) that contributes to cell growth regulation. E2F1: transcription factor that helps the activation of genes involved in DNA synthesis. GF: Growth factors (stimulate the formation of organelles). Cyclins: proteins that drive and regulate the cell cycle (these are fuels). CDKi: inhibitors of cyclin-dependent kinases: these are proteins that inhibit cycle progression (p15-19, p21, p27 and p57). Cdh1: inhibits the progression of mitosis in the phase between metaphase and anaphase, but when dephosphorylated by the cyclin B-CDK1 complex leads to activation of this phase progressing to metaphase-anaphase. G1/S (R point)=restriction point (let it move on to the next phase if the number of organelles was sufficient or check if the cells were in fact well stimulated by the growth factors). G2/M=checks that the genome has been completely replicated and that the cell is large enough to undergo mitosis. M/G1=checks the similar distribution of chromosomes and whether sister chromatids are breaking. Cip/Kip (CDK-interacting protein/cyclin- dependent kinase inhibitor protein).

Reparation of the damage to the DNA

When DNA undergoes endogenous (reactive O2, ROS) or exogenous (e.g. radiation) mutations during the cell cycle, the repair must happen before the next cell division. To this end, 2 serine/tyrosine kinases, ATR (ataxia telangiectasia and Rad3 related) and ATM (ataxia telangiectasia mutated homolog) phosphorylate by activating regulation proteins, CHK1 and CHK2. The first phosphorylates the CDC25 phosphatase, which causes the removal of 2 inhibitory phosphates from CDK1, causing cycle arrest in the S phase or at the G2/M point. The second leads to the activation of the tumor suppressor protein p53 that activates the expression of CDKi p21 and inhibits CDK4, resulting in the blocking of the cycle in the S phase at the G1/S set point (R point) or at the G2/M point, giving the cell time to repair DNA damage. Once the DNA has been successfully repaired, the TMJ and RTA become inactive again. The CHK1 and CHK2 regulation kinases will also be inactivated. CDK1 and CDK4 are no longer inhibited and the cycle continues.[59] On the other hand, if DNA repair fails or the injury is irreparable, the cellular apoptosis cascade is activated. To this end, 2 mechanisms occur in parallel. First, the constant absence of CDC25 causes dimerization and activation of MEK kinase 5 or ASK-1 (apoptosis signal-regulating kinase 1) that activates the cascade resulting in apoptosis. Second, there is an increase in p53 levels that direct the pro-apoptotic protein BAX, and also mediates the permeabilization of the outer mitochondrial membrane (MOMP), resulting in the activation of the apoptosis cascade (Figure 7).[59]



ASK-1= apoptosis signal-regulating kinase 1); p53=p53 protein; BAX=pro-apoptotic protein; MOMP=outer mitochondrial membrane. ATR=, ataxia telangiectasia and related Rad3; ATM=, ataxia telangiectasia mutated homolog); CHK1 and CHK2=regulating proteins, CDC25, CDK1 (cyclin-dependent kinase 1), CDK2 (cyclin-dependent kinase 2), CDKi - p15-19, p21, p27 and p57 (cyclin-dependent kinase inhibitors); DNA=deoxyribonucleic acid

FIGURE 7 — DNA injury and the cell cycle

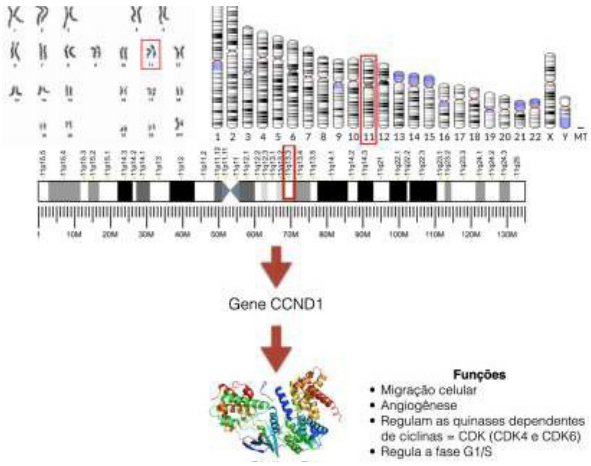
Tumor formation

Any irregularity in the cell cycle predisposes to the formation of carcinomas. Abnormalities in positive regulators (amplification in the CCND1 gene, overexpression of cyclins D1 and their cyclin-dependent kinases - CDK4 and CDK6) accelerate the G1/S phase. Abnormalities in negative regulators (CDKi or underexpression of the p27 protein) fail to inhibit the cycle. This has been demonstrated in several types of cancer such as pancreatic, lung, breast, cervical squamous cell cancer, melanoma, endometrium, lymphoma, multiple myeloma, esophagus, prostate, colon, rectum and thyroid.[51,56,60-64]

Cyclin in papillary thyroid carcinomas (TLC)

It is known that both the dysfunction of type D cyclins, cyclin-dependent kinases (CDK) and cyclin-dependent inhibitory kinases (CDKi) cause interference in the cell cycle and are implicated in the origin of several malignant neoplasms in humans such as breast, squamous carcinomas of the head and neck, esophagus, colon, lung, skin, among others.[62,65] Type D cyclin, discovered in the 1990s, has provided important information in the understanding between extracellular signals and cell cycle proliferation and is subdivided into 3 subtypes (D1, D2 and D3).[58] Cyclin D1, a protein encoded by the CCND1 gene (located on the long arm of chromosome 11, band 11q13.[3)] as shown in Figure 8, is responsible for cell cycle control and coordination. After mitosis, it stimulates it and can also stimulate the resting state cell (G0) to re-enter the cycle.[55,56]

The first studies of cyclin type 1 or cyclin D1 expression in malignant and benign thyroid tissues began in the late nineties.[51,63] Lazzereschi et al.[63] observed that its expression was predominantly cytoplasmic in benign tissues (adenomas) and nuclear in malignant tissues (carcinomas). With these results, the authors concluded that, like other malignant neoplasms, this protein could also be implicated in the tumorigenesis of papillary thyroid carcinomas. The study by Wang et al.[51] analyzed the expression of cyclin D1, cyclin E and cyclin-dependent kinase inhibitor (CDKi) p27 in normal, benign (follicular adenomas) and malignant (papillary carcinomas and follicular variant) thyroid tissues. This study showed that cyclins were not expressed in normal thyroid tissues but were expressed in benign and malignant thyroid tissues. The p27 protein (cell cycle inhibitor) was underexpressed only in carcinomas. Based on these data, these authors concluded that both cyclins and the p27 protein could be implicated in the tumorigenesis of CPT. Cyclins as the first trigger (transforming normal thyroid tissue into adenoma), and the lack of expression of the p27 protein as the second trigger, transforming the adenoma into carcinoma.



Source: Modified by the authors. CDK=cyclin-dependent kinases

FIGURE 8 – Cyclin D1

In a similar model, Saiz et al.[56] studied the labeling of cyclin D1 and E2F-1 proteins in normal thyroid tissues, benign lesions (hyperplastic nodules and adenomas) and malignant lesions (papillary and follicular carcinomas). Both proteins are essential in the regulation of the G1/S transition throughout the cell cycle (cyclin D1 activates the E2F-1 protein). The authors observed overexpression of both proteins in benign and malignant lesions, but not in normal thyroid tissues. Cyclin D1 and E2F-1 protein showed stronger staining in carcinomas, especially in papillary carcinomas. These results allowed the authors to conclude that although these proteins were not useful as differentiators between benign and malignant tissues, they could be implicated in the transformation of normal thyroid follicular cells, first into benign lesions and then into malignant ones, contributing to tumorigenesis.

In another study, Seybt et al.[12] analyzed the immunohistochemical expression of cyclin D1 (intensity and distribution of staining) in papillary thyroid carcinomas (TLC) and its variants, follicular carcinomas (CTC) and follicular adenomas (FA). The authors observed that the intensity of cyclin D1 staining was higher in TLC and its variants compared to CFT ($p=0.013$) and follicular adenomas ($p<0.001$), and the distribution of staining was more diffuse ($p=0.0032$), with no significant difference between CFT and FA. Due to the heterogeneity of the results, the authors did not recommend this marker for diagnosis among DTCs, but evaluated that it could be useful in differentiating between TLC (mainly follicular variant) and follicular adenomas.

The study by Teshima et al.[66,] proposed the immunohistochemical analysis of cyclin D1 (cell proliferation marker, similar to BRAF) in thyroid tissues when lesions are indeterminate in FNA because they present positive predictive values (PPV) and mean nuclear staining rates (NCT) reliable to differentiate papillary carcinomas (PPV = 91.5%, TCN = 48.5%); benign lesions such as follicular adenomas (PPV = 66.7%, TCN = 13.1%) and adenomatous goiters (TCN = 3.4%), with a sensitivity of 94.4% and specificity of 92.3% ($p = 0.003$).

Studies that correlated cyclin D1 with aggressiveness in TLC have also been carried out from the nineties onwards.[4,66-74] Muro-Cacho et al.[74] submitted 35 samples of these tumors and their benign controls (taken from the blocks of adjacent non-neoplastic tissues) to immunostaining with cyclin D1 with subsequent immunohistochemical analysis and clinicopathologic cross-sections, and concluded that staining was predominantly nuclear and more intense in TLC, especially in patients with more advanced tumor staging (AJCC III and IV).

These results were also observed by Khoo et al.[71] who performed PTC immunostaining with cyclin D1 and inhibitory protein p27 to evaluate whether they could be predictors of lymph node metastases in these tumors and also correlated the results with clinical-pathological factors (age, extrathyroid

extension and tumor focality). The authors observed that extrathyroid extension, nuclear overexpression of cyclin D1, and underexpression of p27 protein were strong predictors of lymph node metastases in these carcinomas.

Kovacs et al.[73] comparatively analyzed 15 well-differentiated papillary thyroid carcinomas and 8 micropapillary carcinomas and observed that cyclin D1 was overexpressed in 14 of the 15 carcinomas (93.3%), while in micropapillary carcinomas this proportion was 1 in 8 (12.5%, p = 0.0001). These results allowed the authors to conclude that possibly the benign behavior of most papillary microcarcinomas could be associated with the lack of greater expression of cyclin D1 in these tissues.

Antonaci et al.[67,] combined 2 immunomarkers, cyclin D1 and survivin in TLC, analyzing their respective expressions by immunohistochemistry. They observed that these proteins were overexpressed in tumor tissues and lymph node metastases, suggesting that both proteins could be implicated in the origin of CPT.

Lee et al.[75,] when studying the expressions of cell cycle regulators, cyclin D1, cyclin E, p27 and p57 in tissues of 64 patients with TLC (half with lymph node metastases) and 28 patients with follicular adenoma, observed that the expression of cyclin D1 was significant in the group with TLC and lymph node metastases (p <0.05), suggesting that this protein could be useful in assessing prognosis in these types of carcinomas.

To assess the prognosis of papillary thyroid carcinomas, Jung et al. (2010)[69] submitted 113 cases of papillary thyroid carcinomas with metastases to lymph nodes to cyclin D1 labeling and BRAFV600E mutation testing. While this mutation was not a predictive factor of lymph node metastasis, cyclin D1 was overexpressed in these populations, suggesting that this protein could be used in the identification of papillary thyroid carcinomas with metastatic potential. Other predictive factors in this study were age less than 45 years, tumor size of ≥1 cm, non-follicular variant, lateral tubular growth, extrathyroid extension, and multifocality.

Balta et al.[68,] in a retrospective analysis with 87 patients (TLC = 47; benign lesions = 40), evaluated the prognostic value of oncoprotein expressions (cyclin D1, p53, bcl-2, and c-erbB-2) and observed that both cyclin D1 and p53 protein, because they were overexpressed in samples from patients with TLC, lymph node metastases, and extrathyroidal extension, could have prognostic value in TLC.

In the study Teshima et al.[66,] which analyzed cyclin D1 by immunohistochemistry in indeterminate FNA, showing its validity in the evaluation between benign lesions (follicular adenomas and adenomatous goiters) of papillary carcinomas, it also showed a correlation with tumor aggressiveness, being significantly expressed (p <0.[001)] when analyzing the clinical-pathological variables of TLC (extrathyroid extension, intraglandular and lymph node metastasis).

However, other authors did not find results, similar to the previous ones, not recommending the marker for the study of aggressiveness in TLC.[12,76]

Londero et al.[76] evaluated papillary microcarcinomas (<1 cm), considered a variant of papillary carcinoma, most of which are benign but, in some cases, with a more aggressive course,[70] the cyclin D1 marker was used to try to identify the most aggressive microcarcinomas, but despite having statistically significant results, it failed to recommend the protein as an aggressiveness discriminator in terms of the presence of metastases local-regional or distance due to the great variability of the result

Later, Seybt et al.[12,] although they observed that the expression of cyclin D1 proved useful in differentiating between TLC (mainly follicular variant) and follicular adenomas when they analyzed the expression of this protein in papillary thyroid carcinomas (TLC) and its variants, thyroid follicular carcinomas (FTC) and follicular adenomas (FA), there was no significant difference in the expression of cyclin D1 between FTC and follicular adenomas, nor any feature of cyclin D1 with the nodal status of TLC.

Bartolomei, IJP, et al.[77] in a study with 118 patients with papillary thyroid carcinoma reported that cyclin D1 was expressed in vast majority of carcinomas, being the predominant diffuse distribution. The benign tissue - multinodular goiter - expressed the stain with weak intensity and sparse distribution. Its expression did not correlate with clinicopathological findings (tumor size, angiolymphatic invasion, lymph node metastasis, or distant metastases).

CONCLUSION

The recent literature on aggressive thyroid carcinomas has included many studies on clinical features, pathological subtypes, and molecular features; however, the answer to the question of why some of them evolve with more aggressive pathological features is open in the literature. Advances in biotechnology, molecular research, and genetic studies enable a better understanding of the behavior of these neoplasms. However, the evaluation of the signaling pathways and protein expression of these tumors becomes paramount in the progress for diagnosis, treatment, and prognosis in oncology.

Authors' contributions:
Conceptualization: Ivan José Paredes Bartolomei:
Project administration: Carmen Australia Paredes Marcondes Ribas, Maria Methodology: Augusta Karas Zella
Formal analysis: Ana Maria Waaga-Gasser
Supervision: Martin Gasser
Writing (original draft): Nicolau Gregori Czeczko, Paulo Afonso Nunes Nassif
Writing (proofreading and editing): All authors

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