Molecular Pathways Associated with Aggressiveness of Papillary Thyroid Cancer

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Abstract: The most common thyroid malignancy is papillary thyroid cancer (PTC). Mortality rates from PTC mainly depend on its aggressiveness. Geno- and phenotyping of aggressive PTC has advanced our understanding of treatment failures and of potential future therapies. Unraveling molecular signaling pathways of PTC including its aggressive forms will hopefully pave the road to reduce mortality but also morbidity from this cancer. The mitogen-activated protein kinase and the phosphatidylinositol 3-kinase signaling pathway as well as the family of RAS oncogenes and BRAF as a member of the RAF protein family and the aberrant expression of microRNAs miR-221, miR-222, and miR-146b all play major roles in tumor initiation and progression of aggressive PTC. Small molecule tyrosine kinase inhibitors targeting BRAF-mediated events, vascular endothelial growth factor receptors, RET/PTC rearrangements, and other molecular targets, show promising results to improve treatment of radioiodine resistant, recurrent, and aggressive PTC.

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INTRODUCTION

The most common well differentiated thyroid cancer (TC) is papillary thyroid cancer (PTC) and is usually indolent with a 10 year survival rate of approximately 93\% [1]. Distant metastases at the time of diagnosis are reported in up to 4\% of patients with PTC [2-4]. After surgery patients with PTC are followed by basal and thyroid stimulating hormone (TSH)-stimulated thyroglobulin (Tg) determination, and by neck ultrasonography [5-7]. Histologic variants of PTC can show aggressive features, clinically noted as recurrent TC, resistance to radioactive iodine therapy, propensity to metastasize, and often lower 10 year survival rates, if the aggressive TC is not a microcarcinoma [8]. Interestingly, gene expression profiles of papillary thyroid microcarcinomas may not be different from those of PTCs [9]. Also, familial PTC has a similar clinical and prognostic behaviour, apart from multifocality, when compared with sporadic PTC [10].

Ganly et al. [11] recently reported 100\% 10 year disease specific survival rates for patients with classic PTC and 96\% for those with tall cell variant PTC. In that study, 2.4\% of patients with PTCs with tall cell variant of features developed poorly differentiated or anaplastic TC (ATC). It is important to consider clinico-pathological aspects in combination with molecular features [12-14]. In various geographic areas of the world, different diagnostic criteria for aggressive PTC have been used, leading to discrepancies among pathologists and clinicians. More recently, more consensus regarding the diagnostic criteria of aggressive PTC has been reached [15-17]. Among the most aggressive types of PTC are: diffuse sclerosing variant (accounting of up to 6\% of all PTC), tall cell variant (accounting of up to 11\% of all PTC), and insular TC (less than 1\% of all PTC) [15].

Molecular signaling (or signal transduction) is important for the knowledge of the core biological processes in any type of cancer including TC [18, 19]. The definition of the responses of normal and cancerous cells to environmental and endogenous signals may elucidate the intimate mechanisms at the basis of malignancy formation, progression, invasion and spread to distant metastases. The development of novel anticancer therapies could be allowed by the detailed knowledge of cancer cell signaling [20-28]. However, such data should be used in combination with clinico-pathological data to achieve practical use with hopefully improvement in the care of cancer patients [12-14, 17]. In the last decades, the knowledge about signaling pathways in patients with TC has grown rapidly. One such pathway is the TSH-dependent signaling system.

The thyroid follicular cell, being an endocrine cell, has many “identity-specific” signaling systems, pertinent to the multitude of its endocrine functions and correlated with its status of differentiation. Malignant transformation (e.g., loss of Tg or sodium-iodide symporter [NIS] expression) are associated with specific alterations in these endocrine function-related systems, that usually coexist with derangements in signaling pathways unrelated to the endocrine character. In
this review, we will focus our contribution on aggressive PTC and membrane receptor-associated signaling systems. Intracellular (and nuclear) receptor signaling is also an essential part of cell regulation, as emphasized by the role of the PAX8/peroxisome proliferator-activated receptor (PPAR) γ oncoprotein in follicular thyroid cancer’s (FTC) [29] and the presence of thyroid hormone receptors and functional estrogen in FTC’s and FTC’s which can be stimulated by endocrine disrupting, estrogen mimicking chemicals such as PCB180 and PCP mixtures [30], but we will not comment on this subject.

Herein, we categorize signaling in TC cells that occurs after the activation of plasma membrane receptors and their downstream effector systems, i.e., (1) enzyme-coupled receptors and downstream pathway elements and (2) G-protein coupled receptors (GPCR’s) and associated proteins. Signal sensing and propagation in TC cells are activated by miscellaneous, not yet completely elucidated mechanisms, for example, those responsible for responses of thyrocytes to generic environmental cellular insults (as hypoxia [31] or hydrogen peroxide/reactive oxygen species) [32-35]. Overactivation of pyruvate kinase M2 is necessary for aerobic glycolysis and may provide a selective growth advantage for PTC cells. Reactive oxygen species possibly enhance the phosphatidylinositol-3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) pathways. Hypoxia-inducible factor 1 is regulated by hypoxia and via growth factor signaling pathways including the PI3K pathway [34].

We here review the most important signaling systems operative in TC cells and their interrelationship with other elements that control thyrocyte growth, apoptosis, and differentiation [36] (Fig. 1).

MOLECULAR SIGNALING VIA PLASMA MEMBRANE RECEPTORS IN TC

GPCRs Associated Proteins, and Downstream Effectors

TSH Receptor (TSHR) and G-proteins

TSHR is the par excellence thyroid-specific GPCR, that contains seven-transmembrane domain receptors, and transduces the signal of ambient TSH (its ligand) to the thyrocyte [37]. Upon binding onto TSHR, TSH activates thyroid function, proliferation and differentiation through the activation of both G-protein- and inositol triphosphate (IP3)/phospholipase C (PLC)-dependent pathways.

After thyroidectomy, the suppression of endogenous pituitary TSH production by thyroid hormone treatment in patients with (papillary) TC leads to decreased morbidity and mortality. TSH exposure desensitizes TSHR-dependent signaling through the activation of a G-protein-coupled kinase (GRK), which phosphorylates TSHR [38]. Then, the phosphorylated TSHR attracts arrestins (proteins which inhibit the G-protein dependent signaling cascade) (see below) [39, 40]. If TSHR expression is lost or diminished, as seen in some TCs, TSH does not influence the growth of these tumors [18].

Activating TSHR mutations are rarely identified in TC [41-43].

The downstream delivery of the TSHR signal is achieved via the G-proteins which are submembranar and associated with the TSHR. G-proteins consist of an α–subunit and a βγ-subunit dimer. Gα variant is the predominant type of the α-subunit in thyrocytes. The regulators of G-protein signaling proteins are able to accelerate the hydrolysis of GTP to GDP, also enhancing the kinetics of termination of the hormone signal [44]. From mutations at either of two “hot spots”, i.e. residues Arg201 or Gln227, it can derive hyperactivity of Gα. Gα mutations have been described rarely in TC and in autonomously functioning benign thyroid adenomas [45, 46]. The thyroid manifestations of McCune-Albright syndrome, a sporadic genetic disease caused by a post-zygotic activating mutation of the Gα gene, that include TC (such as clear cell TC), and benign thyroid neoplasms (in particular follicular adenomas), corroborate these observations [47, 48].

TSHR activation also induces stimulation of Gi. Activation of the Gi/G0 system through the stimulation of the P2-purinergic receptor by extracellular adenosine triphosphate induces activation of phospholipase A2 (PLA2) in thyroid FRTL-5 cells [49]. PLA2 hydrolyzes the sn-2 ester bond of cellular phospholipids and produces a free fatty acid and a lyso-phospholipid, which are both lipid signaling molecules; once activated, this signaling pathway is a point of “cross-talk” with pathways activated by mitogens and growth factors (see below). Arachidonic acid (i.e.,5,8,11,14-eicosatetraenoic acid) is the free fatty acid frequently produced, which is the precursor of the eicosanoid family that includes prostaglandins, thromboxanes, leukotrienes and lipoxins [50]. In a model utilizing rat thyroid cells transformed by the RET/PTC oncogenes, Mariggio et al. [51] demonstrated that the control of the cell growth of thyroid tumor is mediated by PLA2 group IV isoform alpha. Arachidonate 5 lipoxygenase expression and activity mediates invasion via metalloproteinase-9 induction in aggressive PTC [52]. Cyclooxygenase-2 expression increases with age and may play a role in aggressive PTC [53].

Regarding other G-proteins in thyroid oncogenesis, Metastatin signaling does not involve Akt/protein kinase B (PKB) [54], and its receptor, Gq/11 -coupled receptor, is overexpressed in PTC, and activates MAPK in the ATC-derived cell line ARO.

GRK regulates the homologous desensitization of different GPCR. The expression of GRK 2,3,5, and 6 can desensitize the TSHR in vitro. GRK 2 expression was found to be decreased in cold thyroid nodules when compared to tissue surrounding the nodules [55].

Protein Kinase A (PKA), Adenylyl cyclase (AC), and cAMP Response Element-binding Protein (CREB)

AC activation via TSHR signaling leads to production of cAMP. Some subsets of TC have increased AC activity, others decreased cAMP production [18]. cAMP activates PKA, which belongs to a large family of proteins, that are heterotetramers, and consist of two regulatory (R-) subunits and two catalytic (C-) subunits. The dissociation of the C-subunits is caused by the binding of cAMP to the R-subunits. Serine and threonine residues of various acceptor proteins are phosphorylated by the catalytic sites on the two dissociated C-subunits, both in the cytoplasm and the nucleus. Inactivating germline mutations of the PKA Rα subunit gene occur in a subgroup of TC and in a multiple neoplasia syndrome, called Carney complex [56, 57].
PKA phosphorylates several substrates, as the p85 phosphoprotein, in thyrocytes, thus leading to enhancement of the interaction between p21/Ras and PI3K. At the same time, cAMP can also inhibit Raf1 kinase signaling decreasing Raf1 availability to Ras. Once activated, PKA also phosphorylates other proteins, as CREB, which is a nuclear transcription factor belonging to the large family of leucine zipper DNA binding proteins and binds to cAMP response elements. In TC, CREB expression is strongly downregulated, but probably not related to the functional state of differentiation of the malignancy, as evaluated by NIS expression levels [58]. Praja2, a widely expressed really interesting new gene ligase, degrades the regulatory subunits of PKA, thereby controls the strength and duration of PKA signaling in response to cAMP. Praja2 is markedly overexpressed in differentiated TC and shows an inverse correlation with the malignant phenotype of the tumor [59].

Protein kinase C (PKC) and PLC

TSHR activation stimulates pathways which depend on the PLC-γ, via coupling of the receptor to members of the Gq/11 family [60]. The activity of the plasma membrane PLC is increased in neoplastic thyroid tissue, correlating to the degree of tumoral de-differentiation [61]. PLC stimulation causes the hydrolysis of phosphatidylinositol 4,5-bisphosphate, which generates diacylglycerol (DAG) and IP3. IP3 increases the concentration of intracellular ionized calcium (iCa++) permitting its release from the endoplasmic reticulum, while DAG activates PKC, which in turn, under certain conditions, can also be activated directly by iCa++, and phosphorylates several target proteins [62]. Usually, the activation of the PKA pathway antagonizes the PI3-iCa++-PKC pathway in thyroid tissue.

Enzyme-coupled Membrane Receptor Systems

The Receptor-tyrosine Kinases (RTK)’s and Their Downstream Effectors

RTK’s are membrane receptors with intrinsic tyrosine kinase (TK) activity. These receptors phosphorylate downstream intracellular substrate proteins or can undergo autophosphorylation [63]. The elements of the Ras – MAPK pathway, PI3K-PKB/Akt system, PLC-γ, and RAS/GTPase-activating proteins are downstream molecules along RTK-dependent pathways [19, 64].
Insulin-like growth factor-1 increases the expression of vascular endothelial growth factor (VEGF) in TC cells, through both AP-1/hypoxia inducible factor-1α- and PI3K-dependent mechanisms [65]. High expression of VEGF in TC seems to correlate with an increased risk of recurrence, lymph node metastases, and advanced tumor stage [66]. Neuruplin-2 is a coreceptor for VEGF-D and correlates with lymph node metastases in PTC [67].

Epidermal growth factor (EGF) activates the PKC pathway. Overexpression of the EGF receptor in BRAF wild-type PTC may represent a biomarker for aggressive PTC [68]. Of note, another growth factor family member, platelet derived growth factor (PDGF) receptor B is upregulated in clinically aggressive PTC [69]. The effects of PDGF ligands are influenced by inflammatory cytokines. Interleukin (IL)-6 expression is related with aggressiveness in PTC [70]. Interestingly, concomitant Hashimoto’s thyroiditis in patients with PTC seems to correlate, with low recurrence and less aggressive clinical stage, whereas Graves’s disease is associated with larger and multifocal TC [43, 71]. Chemokines and their receptors appear to be involved in several steps of tumorigenesis and progression in TC. Oncogenes can upregulate chemokine (C-X-C) motif ligand 10 (CXCL10), thereby further stimulating proliferation and invasion. PPARγ thiazolidinediones can inhibit growth of PTC by inhibiting CXCL10 secretion in thyroid follicular cells [72]. C-X-C chemokine receptor 4 (CXCR4) expression and BRAF mutation status may in concert lead to a more aggressive phenotype in PTC [73]. Young patients with PTC with intense CD-40 ligand expression often have aggressive, multifocal, and recurrent TC [74]. The frequency of regulatory T cells and tumor associated lymphocytes in primary PTC seems to correlate with more aggressive disease [75]. The prevalence of nuclear location of CD30L and the proportion of CD30L+ cells are involved in aggressiveness of PTC with CD30L/CD30 signaling being activated especially in an autocrine fashion [76].

The nuclear factor kappa-B (NF-kB) proteins, transcription factors, have an important role in human malignancies including TC by controlling proliferative and antiapoptotic signaling pathways. Oncogenic proteins such as RET/PTC, RAS, and BRAF, can induce NF-kB activation in TC. NF-kB inhibitors can induce antiproliferative effects and apoptosis and may be of great benefit in aggressive TC [77]. RET/PTC3 is markedly prevalent in PTC induced by radiation, heat, ultraviolet light, and osmotic shock, can also depend on RAS and selected RTK’s [85]. Cytokines and other inflammatory mediators can activate the p38 MAPK kinase pathway. Ribosome protein S6 kinase-B, nuclear proteins, as CREB and activating transcription factor are the final effectors of p38 MAPK signaling [86].

The Raf kinases (i.e. Raf-1 and b-Raf) are downstream from RAS. BRAF is a member of the Raf protein family and activates the MAPK pathway. Earlier studies have shown a correlation between BRAF mutation V600E and aggressiveness of PTC but recent studies have challenged this concept, suggesting that BRAF mutation in PTC is a later subclonal event and that no correlation between BRAF-positive primary focus of papillary microcarcinoma and more aggressive or recurrent disease exists [64, 87-89].

RTK’s can activate not only the RAS-MAPK pathway, but also PI3K signaling, leading to the generation of D-3-phosphoinositides, as phosphatidylinositol-3,4,5-trisphosphate. The role of this pathway in TC has first been recognized when patients with Cowden syndrome caused by a germline mutation in the PTEN gene were found to have FTC. Increased expression and activation of Akt has been demonstrated to activate the P13/akt pathway. In this setting, Akt-1 and Akt-2 are the most important genes [19, 90-92]. Activation of Akt leads to a phosphorylation cascade with involvement of multiple proteins, including mammalian target of rapamycin (mTOR), and ultimately to increased cell growth and reduced apoptosis. Rapamycin, which is a strong immunosuppressant, inhibits IL-2 dependent T-cell proliferation. mTOR signaling is activated in aggressive PTC [93]. The occurrence of multiple tumors including PTC in one patient should make one consider a hereditary tumor syndrome [94, 95].
The TK-associated Receptors (TKAR’s) and Their Downstream Effectors

TKAR’s represent a large family of receptors, lacking an intrinsic TK domain, but able to activate different intracellular TK’s after ligand binding, thus leading indirectly to the phosphorylation of downstream target substrates.

Cytokine receptors activation leads to phosphorylation and activation of Janus kinases (Jak), which subsequently phosphorylate and activate differentially members of the signal transducer and activator of transcription (STAT) proteins, leading to the regulation of transcription of specific genes, along the cascade of theJak-STAT pathway [18].

IL-6 and its receptor IL-6R have been evidenced in less than 50% of PTC studied [96]. STAT-3, the downstream effector of the IL-6R system, appeared much better correlated with the expression of HGFR (MET) in these PTC [96].

The members of the tumor necrosis factor receptor family are closely related to TKAR’s. These heterotrimeric receptor systems are also collectively known as “death receptors” as they are responsible for induction of apoptosis (or programmed cell death). The caspase recruitment domain is part of the downstream effectors of the death receptor-dependent signaling cascade. Caspases constitute the common final pathway of all apoptotic signals, leading to cell lysis [97]. Fas (or CD95/Apo-1), one of these “death receptors”, in concert with its ligand (FasL) initiates a number of processes through the activation of caspases, along the pathway of cell death [98]. The antiapoptotic protein survivin plays a role in many human cancers and tumors [99]. The upregulation of survivin expression has been suggested as a marker of dedifferentiation or aggressiveness in PTC [100]. Similarly, FasL correlates with a more aggressive phenotype of PTC, when expressed in high levels [101]. Apoptosis could ensue either through infiltrating cytotoxic lymphocytes or as part of the natural course of rapidly growing tumors outstripping their growth resources, in TC.

The Serine-threonine Kinase (STK) Receptors and Their Downstream Effectors

The ligands for these receptors are members of the transforming growth factor-β (TGF-β) superfamily. The STK receptor-dependent signaling systems have an essential role during embryonic development, and participate in adult tissue homeostasis [102]. The expression of the ligands belonging to this family, and their cognate receptors, including TGF-β, activin, and bone morphogenetic proteins, has been identified in both normal and neoplastic thyroid tissues [18, 103].

The “downstream” signal transduction cascade includes the phosphorylation and thus the activation of cytoplasmic small mother against decapentaplegic (SMAD) proteins (SMAD-1 to -8). It is believed that the mechanism of TGF-β-induced growth inhibition (eventually leading to apoptosis) in normal thyrocytes involves reduction in the levels of p27kip1 (a cyclin-dependent kinase [cdk] inhibitor), which is overridden in malignant thyrocytes by NF-xB activation [104]. Overexpression (immunohistochemically) of cell cycle regulator cyclin D1 and estrogen receptor β and underexpression of p27 may predict lymph node metastases in PTC [105, 106].

MicroRNAs

Small noncoding RNA genes composed of 21 to 25 nucleotides, called microRNAs, can inhibit expression levels of genes at the transcriptional and posttranslational levels. In PTC, miR-221, miR-222, and miR-146b have been identified as the most upregulated miRNAs and strongly correlate with extrathyroidal invasion and drug resistance [18, 107, 108]. miR-1 silences chemokine CXCR4, which is important for lymph node metastases. Through cyclin D1, miR-31 and miR-130b regulate proliferation, apoptosis, and cell cycle [109].

Potential Molecular Targets for the Treatment of Aggressive PTC

Considering the high frequency of BRAF mutations in PTC, the development of BRAF inhibitors is appealing. The agent BAY43-9006 not only inhibits mutant V600E BRAF but also FLT-3, c-KIT, VEGFR-2, VEGFR-3, and PDGFRβ kinases [19]. Lenvatinib is a TK inhibitor targeting FGFR1-4, RET, VEGFR1-3, KIT, and PDGFRβ with response rates around 50%. Various other agents have completed phase II and/or III studies and are listed and reviewed in [19]. Clinically meaningful increases in iodine uptake and retention in subgroups of patients with thyroid cancer that is (initially) refractory to radioiodine, can occur with certain agents such as MAPK kinase 1 and 2 inhibitor selumetinib, perhaps with greater effectiveness if TC is RAS mutant (BRAF) positive [28]. A recent meta-analysis suggested a modest effect of the VEGF-targeted therapy with sorafenib in patients with radioiodine-refractory differentiated TC, while it showed a potential therapeutic effect in Chinese patients with PTC and radioiodine refractory pulmonary metastases [110, 111].

SUMMARY

One of the most well studied endocrine neoplasms at the molecular level is PTC. As the mortality rates for TC mainly depend on its aggressiveness including ineffectiveness of surgical and radioiodine therapy, it is critical to understand the molecular pathobiology of cancer cell signaling of this “model malignancy”, considering that PTC has many ligands, cognate receptors, and downstream effectors.

The growing knowledge of the elements of these systems and the interaction between them and other constituents of the cell proliferation machinery (protein synthesis and degradation, cell cycle, gene transcription) has led to novel therapies including the use of vandetanib, motesanib, axitinib, cabozantinib, sorafenib, sunitinib, pazopanib, lenvatinib, selumetinib, and others in treating patients with progressive TC of medullary, papillary and follicular subtypes.

Hopefully, molecular profiling of (aggressive papillary) TC will help identify patients with advanced TC who could benefit from new treatment modalities. MAPK kinase inhibitors such as selumetinib increase the effectiveness of radioiodine therapy, particularly in patients with RAS mutations [3, 27].

Future trials will likely involve combined therapeutics and targets to overcome drug resistance [112]. The challenge
of any future TC therapy will remain to unravel cooperative pathways that influence tumorigenesis and tumor progression, even in individuals of families with identical genetic germline defects and/or environmental risk factors [113, 114].

CONFLICT OF INTEREST

The author SB has nothing to declare. CAK has served on the Advisory Board for Bayer Pharmaceuticals.

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ABBREVIATIONS

PTC = Papillary thyroid cancer
TC = Thyroid cancer
TSH = Thyroid stimulating hormone
Tg = Thyroglobulin
ATC = Anaplastic TC
NIS = Sodium-iodide symporter
PPARγ = Peroxisome proliferator-activated receptor
FTC = Follicular thyroid cancer
MAPK = Mitogen-activated protein kinase
PI3K = Phosphatidylinositol-3-kinase
TSHR = TSH receptor
IP3 = Inositol triphosphate
PLC = Phospholipase C
GRK = G-protein-coupled kinase
PLA2 = Phospholipase A2
PKB = Protein kinase B
AC = Adenyl cyclase
PKA = Protein kinase A
CREB = cAMP response element-binding protein
PKC = Protein kinase C
DAG = Diacylglycerol
iCa++ = Intracellular ionized calcium
RTK’s = Receptor-tyrosine kinases
TK = Tyrosine kinase
VEGF = Vascular endothelial growth factor
EGF = Epidermal growth factor
PDGF = Platelet derived growth factor
IL = Interleukin
CXCL10 = Chemokine (C-X-C) motif ligand 10
CXCR4 = C-X-C chemokine receptor 4
NF-kB = Nuclear factor kappa-B
FGFR = Fibroblast growth factor receptor
HGFR = Hepatocyte growth factor receptor
VEGFR = VEGF receptor
mTOR = Mammalian target of rapamycin
TKAR’s = TK-associated receptors
JAK = Janus kinases
STAT = Signal transducer and activator of transcription
IL-6R = IL-6 receptor
STK = Serine-threonine kinase
TGF-β = Transforming growth factor-β
SMAD = Small mother against decapentaplegic

REFERENCES

[1] Carcangiu, M.L.; Zampi, G.; Pupi, A.; Castagnoli, A.; Rosai, J. Papillary carcinoma of the thyroid. A clinicopathologic study of 241 cases treated at the University of Florence, Italy. Cancer, 1985, 55(4), 805-828.
[2] Hefer, T.; Joachims, H.Z.; Hashmonai, M.; Ben-Ariei, Y.; Brown, J. Highly aggressive behavior of occult papillary thyroid carcinoma. J. Laryngol. Otol., 1995, 109(11), 1109-1112.
[3] Reiners, C. Thyroid cancer in 2013: Advances in our understanding of differentiated thyroid cancer. Nat. Rev. Endocrinol., 2014, 10(2), 69-70.
[4] Sabra, M.M.; Dominguez, J.M.; Grewal, R.K.; Larson, S.M.; Ghossein, R.A.; Tuttle, R.M.; Fagin, J.A. Clinical outcomes and molecular profile of differentiated thyroid cancers with radioiodine-avid distant metastases. J. Clin. Endocrinol. Metab., 2013, 98(5), E829-836.
[5] Antonelli, A.; Miccoli, P.; Fallahi, P.; Grosso, M.; Nesti, C.; Spinelli, C.; Ferrannini, E. Role of neck ultrasonography in the follow-up of children operated on for thyroid papillary cancer. Thyroid, 2003, 13(5), 479-484.
[6] Spinelli, C.; Bertocchini, A.; Antonelli, A.; Miccoli, P. Surgical therapy of the thyroid papillary carcinoma in children: experience with 56 patients < 16 years old. J. Pediatr. Surg., 2004, 39(10), 1500-1505.
[7] Antonelli, A.; Miccoli, P.; Derzhitski, V.E.; Panasiuk, G.; Solovieva, N.; Baschieri, L. Epidemiologic and clinical evaluation of thyroid papillary microcarcinoma. J. Clin. Endocrinol. Metab., 2004, 90(5), 323-329.
[8] Kuo, E.J.; Goffredo, P.; Sosa, J.A.; Roman, S.A. Aggressive variants of papillary thyroid microcarcinoma are associated with extrathyroidal spread and lymph-node metastases: a population-level analysis. Thyroid, 2013, 23(10), 1305-1311.
[9] Kim, H.Y.; Park, W.Y.; Lee, K.E.; Park, W.S.; Chung, Y.S.; Cho, S.J.; Youn, Y.K. Comparative analysis of gene expression profiles of papillary microcarcinoma and papillary thyroid carcinoma. J. Cancer. Res. Ther., 2010, 6(4), 452-457.
[10] Pinto, A.E.; Silva, G.L.; Henrique, R.; Menezes, F.D.; Teixeira, M.R.; Leite, V.; Cavaco, B.M. Familial vs. sporadic papillary thyroid carcinoma. A clinicopathologic study of 241 cases treated at the University of Florence, Italy. Cancer, 1985, 55(4), 805-828.
[11] Kim, H.Y.; Park, W.Y.; Lee, K.E.; Park, W.S.; Chung, Y.S.; Cho, S.J.; Youn, Y.K. Comparative analysis of gene expression profiles of papillary microcarcinoma and papillary thyroid carcinoma. J. Cancer. Res. Ther., 2010, 6(4), 452-457.
[12] Nienmeier, L.A.; Kuffner, A.H.; Song, C.; Carty, S.E.; Hodak, S.P.; Yip, L.; Ferris, R.L.; Tseng, G.C.; Seethala, R.R.; Lebeau, S.O.; Destarte, M.T.; Conte, J.; Johnson, J.T.; Stewart, A.F.; Nikiforov, Y.E. A combined molecular pathologic score improves risk stratification of thyroid papillary microcarcinoma. Cancer, 2012, 118(8), 2069-2077.
[13] Bansal, M.; Gandhi, M.; Ferris, R.L.; Nikiforov, M.N.; Yip, L.; Carty, S.E.; Nikiforov, Y.E. Molecular and histopathologic charac-
teristics of multifocal papillary thyroid carcinoma. Am. J. Surg. Pathol., 2013, 37(10), 1586-1591.

[14] Miccoli, P.; Miccoli, M.; Antonelli, A.; Minuto, M.N. Clinico-pathologic and molecular disease prognostication for papillary thyroid cancer. Expert. Rev. Anticancer. Ther., 2009, 9(9), 1261-1275.

[15] Roman, S.; Sosa, J.A. Aggressive variants of papillary thyroid carcinoma. Curr. Opin. Oncol., 2013, 25(1), 33-38.

[16] Kleiman, D.A.; Beninati, T.; Soni, A.; Shou, Y.; Zarnegar, R.; Fahey, T.J. 3rd. Does betaless category predict aggressive features in malignant thyroid nodules? Ann. Surg. Oncol., 2013, 20(11), 3484-3490.

[17] Asiloi, S.; Erickson, L.A.; Sebo, T.J.; Zhang, J.; Jin, L.; Thompson, G.B.; Lloyd, R.V. Papillary thyroid carcinoma with prominent hobo-nail features; a new aggressive variant of moderately differentiated papillary carcinoma. A clinicopathologic, immunohistochemi-cal, and molecular study of eight cases. Am. J. Surg. Pathol., 2010, 34(1), 44-52.

[18] Sarlis, N.J.; Benvenga, S. Molecular signaling in thyroid cancer. Can. Treat. Res., 2004, 122 237-264.

[19] Omur, O.; Baran, Y. An update on molecular biology of thyroid cancers. Crit. Rev. Oncol. Hematol., 2013, 99(3), 233-252.

[20] Benvena, S. Emerging therapies in sight for the fight against de-differentiated thyroid cancer. J. Clin. Endocrinol. Metab., 2011, 96(2) 47-50.

[21] Antonelli, A.; Fallahi, P.; Ferrari, S.M.; Carpi, A.; Berti, P.; Materazzi, G.; Minuto, M.; Guastalli, M.; Miccoli, P. Dedifferentiated thyroid cancer: a therapeutic challenge. Biomed. Pharmacother., 2008, 62(8), 559-563.

[22] Antonelli, A.; Bocci, G.; La Motta, C.; Ferrari, S.M.; Fallahi, P.; Fioravanti, A.; Sartini, S.; Minuto, M.; Piaggi, S.; Corti, A.; Ali, G.; Fontanini, G.; Danesi, R.; Da Settimo, F.; Miccoli, P. Novel pyrazolopyrimidine derivatives as tyrosine kinase inhibitors with antitumoral activity in vitro and in vivo in papillary dedifferentiated thyroid cancer. J. Clin. Endocrinol. Metab., 2011, 96(2), E288-E296.

[23] Antonelli, A.; Bocci, G.; La Motta, C.; Ferrari SM; Fallahi, P.; Ruffilli I; Di Domenicantonio A; Fioravanti A; Sartini S; Minuto M; Piaggi S; Corti A; Ali G; Desidero T; Berti P; Fontanini G; Danesi R; Da Settimo F; Miccoli P. CLM94, a novel cyclic amide with anti-VGFR-2 and antiangiogenic properties, is active against primary anaplastic thyroid cancer in vitro and in vivo. J. Clin. Endocrinol. Metab., 2012, 97(4), E528-E536.

[24] Antonelli, A.; Bocci, G.; Fallahi, P.; La Motta, C.; Ferrari, S.M.; Mancusi, C.; Fioravanti, A.; Di Desidero, T.; Sartini, S.; Corti, A.; Piaggi, S.; Materazzi, G.; Spinelli, C.; Fontanini, G.; Danesi, R.; Da Settimo, F.; Miccoli, P. CLM3, a multitarget tyrosine kinase inhibitor with antiangiogenic properties, is active against primary anaplastic thyroid cancer in vitro and in vivo. J. Clin. Endocrinol. Metab., 2014, 99(4), E572-81.

[25] Giunti, S.; Antonelli, A.; Antos rose, A.; Santaripa, L. Cellular signaling pathway alterations and potential targeted therapies for medullary thyroid carcinoma. Int. J. Endocrinol., 2013, 2013, 803171.

[26] Antonelli, A.; Fallahi, P.; Ferrari, S.M.; Ruffilli, I.; Santini, F.; Minuto, M.; Galleri, D.; Miccoli, P. New targeted therapies for thyroid cancer. Curr. Genomics., 2011, 12(8), 626-631.

[27] Wells, S.A. Jr; Santoro, M. Update: the status of clinical trials of kinase inhibitors in thyroid cancer. J. Clin. Endocrinol. Metab., 2014, 99(5), 1543-55.

[28] Ho, A.L.; Grewal, R.K.; Leboeuf, R.; Sherman, E.J.; Pfister, D.G.; Deandreis, D.; Pentlow, K.S.; Zannonicco, P.B.; Haque, S.; Gavane, S.; Ghossein, R.A.; Ricarte-Filho, J.C.; Dominguez, J.M.; Shen, R.; Tuttle, R.M.; Larson, S.M.; Fagin, J.A. Selumetinib-enhanced adenylate cyclase activity and growth of these tumors. J. Clin. Endocrinol. Metab., 2013, 98(7), 623-632.

[29] Placzkowski, K.A.; Reddi, H.V.; Greve, S.K.; Eberhardt, N.L.; McVler, B. The role of the PAX8/PAPagama fusion oncogene in thyroid cancer. PPAR Res., 2008, 2008, 278229.

[30] Vandenbergh, L.N.; Colborn, T.; Hayes, T.B.; Heindel, J.J.; Jacobs, D.B.; Jr; Lee, D.A.; Shioda, T.; Soto, A.M.; von Were solsh, W.V.; Zoellér, R.T.; Myers, J.P. Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. Endocr. Rev., 2012, 33(3), 378-455.

[31] Kiang, J.G.; Wang, X.D.; Ding, X.Z.; Gist, I.D.; Smallridge, R.C. Heat shock inhibits the hypoxia-induced effects on iodide uptake and signal transduction and enhances cell survival in rat thyroid FRTL-5 cells. Thyroid., 1996, 6(5), 475-483.
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regulation of archidonic acid mobilization. *FEBS Lett.*, 2002, 531(1), 2-6.

Mariggiò, S. Filippi BM, Iurisci C, Dragni LK, De Falco V, Santoro M, Corda D. Cytosolic phospholipase A2 alpha regulates cell growth in RET/PTC-transformed thyroid cells. *Cancer Res.*, 2007, 67(14), 11769-11778.

Kummer, N.T.; Nowickim, T.S.; Azzi, J.P.; Reyes, I.; Iacob, C.; Xie, S.; Swati, I.; Darzynekiewicz, Z.; Gottinger, K.H.; Suslina, N.; Schantz, S.; Tiwari, R.K.; Gelbier, J. Arachidonic acid 5-lipoxygenase expression in papillary thyroid carcinoma promotes invasion via MMP-9 induction. *J. Cell. Biochem.*, 2012, 113(6), 1998-2008.

Siironen, P.; Ristimäki, A.; Louhimo, J.; Haapiainen, R.; Haglund, C. Expression of COX-2 is increased with age in papillary thyroid cancer. *Histopathology*, 2004, 44(5), 490-497.

Ringel, M.D.; Hardy, E.; Bernet, V.J.; Burch, H.B.; Schuppert, F.; Burman, K.D.; Saji, M. Metastin receptor is overexpressed in papillary thyroid cancer and activates MAP kinase in thyroid cancer cells. *J. Clin. Endocrinol. Metab.*, 2002, 87(5), 2399.

Voigt, C.; Holzapfel, H.P.; Paschke, R. Decreased expression of G-protein coupled receptor kinase 2 in cold thyroid nodules. *Exp. Clin. Endocrinol. Diabetes.*, 2005, 113(2), 102-106.

Kirschners, L.S.; Carney, J.A.; Pack, S.D.; Taymans, S.E.; Giatrakos, C.; Cho, Y.S.; Cho-Chung, Y.S.; Stratakis, C.A. Mutations of the gene encoding the protein kinase A type 1-alpha regulatory subunit in patients with the Carney complex. *Nat. Genet.*, 2000, 26(1), 89-92.

Sandrinis, F.; Frangi, L.; Sarfis, N.J.; Kirschner, L.S.; Faridakis, C.; Gimm, O.; Stratakis, C.A. Regulatory subunit type 1-alpha of protein kinase A (PRKAR1A): a tumor-suppressor gene for sporadic thyroid cancer. *Genes. Chromosomes. Canc.*, 2002, 35(2), 182-192.

Ruggeri, R.M.; Villari, D.; Simonne, A.; Scarfi, R.; Attard, M.; Orlandi, F.; Barresi, G.; Trimarchi, F.; Trovato, M.; Benvenega, S. Co-expression of interleukin-6 (IL-6) and interleukin-6 receptor (IL-6R) in thyroid nodules is associated with co-expression of CD30 ligand/CD30 receptor. *J. Endocrinol. Invest.*, 2002, 25(11), 869-875.

Huang, B.Y.; Hseuh, C.; Chao, T.C.; Lin, K.J.; Lin, J.D. Well-differentiated thyroid carcinoma with concomitant Hashimoto's thyroiditis presents with less aggressive clinical stage and low recurrence. *Endocr. Pathol.*, 2011, 22(3), 144-149.

Antonelli, A.; Ferrari, S.M.; Fallahi, P.; Frascella, S.; Piaggi, S.; Gelmini, S.; Lupi, C.; Minucci, M.; Berti, P.; Benvenega, S.; Basolo, F.; Orlando, C. Micolli, P. Dysregulation of secretion of CXCL alpha-chemokine CXCL10 in papillary thyroid cancer: modulation by peroxisome proliferator-activated receptor-gamma agonists. *Endocr. Relat. Canc.*, 2009, 16(4), 1299-1311.

Torregrossa, L.; Giannini, R.; Borelli, N.; Sensi, E.; Melillo, R.M.; Locatelli, P.; Materazzi, G.; Miccoli, P.; Santoro, M.; Basolo, F. CXC/CR4 expression correlates with the degree of tumor infiltration and BRAF status in papillary thyroid carcinomas. *Mod. Pathol.*, 2012, 25(1), 46-55.

Costello, A.; Rey-Hipolito, C.; Patel, A.; Oakley, K.; Vasco, V.; Calabria, C.; Tuttle, R.M.; Francis, G.L. Thyroid cancers express CD40 and CD40 ligand: cancers that express CD40 ligand may have a greater risk of recurrence in young patients. *Thyroid*, 2005, 15(2), 105-113.

French, J.D.; Weber, Z.J.; Toretwell, D.L.; Said, S.; Klopoper, J.P.; Haugen, B.R. Tumor-associated lymphocytes and increased Foxp3+ regulatory T cell frequency correlate with more aggressive papillary thyroid cancer. *J. Clin. Endocrinol. Metab.*, 2010, 95(5), 2325-2333.

Trovato, M.; Villari, D.; Ruggeri, R.M.; Quattrocorici, E.; Fogatta, F.; Simone, A.; Scarfi, R.; Magro, G.; Batolo, D.; Trimarchi, F.; Benvenega, S. Expression of CD30 ligand and CD30 receptor in normal thyroid and benign and malignant thyroid nodules. *Thyroid.*, 2001, 11(7), 621-628.

Li, X.; Abdel-Mageed, A.B.; Mondal, K.; Kandil, E. The nuclear factor kappa-B signaling pathway as a therapeutic target against thyroid cancers. *Thyroid.*, 2012, PMID: 22889272.

Romei, C.; Elisei, R. RET/PTC Translocations and Clinico-Pathological Features in Human Papillary Thyroid Carcinoma. *Front Endocrinol. (Lausanne)*, 2012, 3, 54.

Sugishita, Y.; Kammori, M.; Yamada, O.; Poon, S.S.; Kobayashi, M.; Onoda, N.; Yamazaki, K.; Fukumori, T.; Yoshikawa, K.; Onose, H.; Ishii, S.; Yamada, E.; Yamada, T. Amplification of the human epidermal growth factor receptor 2 gene in differentiated thyroid carcinomas predicts metastatic disease. *Virchows Arch.*, 2003, 442(4), 322-328.

Trovato, M.; Villari, D.; Ruggeri, R.M.; Mose, M.; Bartolone, L.; Spinella, S.; Simone, A.; Scarfi, R.; Magro, G.; Batolo, D.; Trimarchi, F.; Benvenega, S. Expression of CD30 ligand and CD30 receptor in normal thyroid and benign and malignant thyroid nodules. *Thyroid.*, 2001, 11(7), 621-628.

Kremser, R.; Ohrist, P.; Spitzer, G.; Erler, H.; Kendler, D.; Kneissl, M.; Inagaki, M.; Santoro, M.; Basolo, F. HER2/Neu overexpression in differentiated thyroid carcinomas predicts metastatic disease. *Virchows Arch.*, 2003, 442(4), 322-328.

Trovato, M.; Villari, D.; Bartolone, L.; Spinella, S.; Simonne, A.; Voli, M.A.; Trimarchi, F.; Batolo, D.; Benvenega, S. Expression of the hepatocyte growth factor receptor 2 gene in differentiated thyroid cancer correlates with telomere shortening. *Int. J. Oncol.*, 2013, 42(5), 1589-1596.

Kremser, R.; Ohrist, P.; Spitzer, G.; Erler, H.; Kendler, D.; Kneissl, M.; Inagaki, M.; Santoro, M.; Basolo, F. HER2/Neu overexpression in differentiated thyroid carcinomas predicts metastatic disease. *Virchows Arch.*, 2003, 442(4), 322-328.

Trovato, M.; Villari, D.; Bartolone, L.; Spinella, S.; Simonne, A.; Voli, M.A.; Trimarchi, F.; Batolo, D.; Benvenega, S. Expression of the hepatocyte growth factor and c-met in normal thyroid, non-neoplastic, and neoplastic nodules. *Thyroid.*, 1998, 8(2), 125-131.

Ruggeri, R.M.; Vitelli, R.; Benvenega, S.; Trimarchi, F.; Benvenega, S.; Trovato, M. HGF/C-MET system pathways in benign and malignant thyroid nodules. *Thyroid.* 2005, 15(2), 105-113.

Kumar, S.; Boehm, J.; Lee, J.C. p38 MAP kinases: key signalling molecules as therapeutic targets for inflammatory diseases. *Nat. Rev. Immunol.*, 2003, 3, 151-161.
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