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Review

Pathogenesis of Thyroid Cancer with Particular Emphasis on the Role of Anoikis

Akın Kağızmanlı G et al. Pathogenesis of Thyroid Cancer and Anoikis

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Abstract
Thyroid cancer (TC) is the most prevalent malignancy of the endocrine system, with its incidence has been increasing worldwide in recent years. Although it generally has a favorable prognosis, aggressive forms such as anaplastic TC are associated with high mortality rates. Cell-microenvironment interactions largely influence the progression and metastatic behavior of TC; however, the precise mechanisms underlying these processes remain inadequately understood. One critical factor influencing metastasis is anoikis, a form of programmed cell death triggered by the detachment of cells from the extracellular matrix. Resistance to anoikis allows tumor cells to escape apoptosis, survive in circulation, and metastasize to distant organs. Given its pivotal role in metastasis, anoikis resistance represents a key area of study in understanding cancer progression. This review covers the molecular mechanisms and pathogenesis of TC, particularly emphasizing the role of anoikis resistance in metastatic spread and its potential as a therapeutic target.
Keywords: Thyroid cancer, anoikis, metastasis, apoptosis, extracellular matrix

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Introduction

Thyroid cancer (TC) is the most prevalent malignancy of the endocrine system, and its incidence has been increasing worldwide in recent years (1,2). While it can affect both sexes, approximately 75% of TC cases occur in women. TC is most commonly diagnosed in individuals in their early 50s but can occur at any age. Notably, TC is the most common malignancy among adolescents and young adults aged 16-33 years (1). In the pediatric population specifically, TC constitutes over 6% of all childhood cancers, with its occurrence showing a marked rise over the past four decades (3,4).

TCs originate from the follicular and parafollicular C cells of the thyroid gland and are histologically divided into three main categories (1,5). The most prevalent among them, accounting for over 95% of all TC cases, are differentiated thyroid carcinomas (DTCs) derived from follicular epithelial cells. These include papillary thyroid cancer (PTC), follicular thyroid cancer (FTC), and Hurtle cell thyroid cancer (1). PTC generally progresses slowly and is associated with a favorable prognosis, although it frequently presents with multifocal lesions and regional lymph node metastases due to its lymphatic spread (6). On the other hand, FTC demonstrates a more invasive growth pattern and is often characterized by capsule formation. This type is more commonly seen in adults and rare in childhood. Cases with only capsular invasion typically have a favorable prognosis, whereas vascular invasion may lead to metastases in distant organs. Hurthle cell tumors, a rare subtype of DTCs, account for 5% of all cases. These tumors are more frequently seen in men, typically present as encapsulated masses, and predominantly occur in elderly individuals (2,6,7). Notably, compared to adult patients, children and adolescents with DTCs are more prone to regional lymphatic spread, extension beyond the thyroid capsule, and distant metastases, despite often responding well to treatment (8).

In contrast to DTCs, poorly differentiated thyroid carcinomas (PDTCs) represent a more aggressive cancer type, also originating from follicular cells. Histopathologically and biologically, they demonstrate characteristics intermediate between well-differentiated carcinomas (e.g., PTC and FTC) and anaplastic carcinoma. The undifferentiated tumor arising from follicular cells is anaplastic thyroid cancer (ATC), the rarest (<1%) type, and is characterized by a highly aggressive course and a poor prognosis (1,5). Medullary thyroid cancer (MTC), which differs from the aforementioned subtypes, originates from parafollicular C cells and constitutes approximately 2-3% of all TCs. While 70% of MTC cases occur sporadically, 30% are associated with Multiple Endocrine Neoplasia Type 2 (MEN2) syndrome and are inherited in an autosomal dominant manner (1,6,7,9).

Significant advancements have been made in understanding the molecular and clinical characteristics of TC, yet important gaps remain, particularly in identifying the factors that drive differences in aggressiveness and metastatic potential among its subtypes. Anoikis resistance, a process that allows tumor cells to escape apoptosis after detachment from the extracellular matrix, is an important mechanism in metastasis. However, its involvement in TC metastasis is not well understood, and studies on this phenomenon in TC are limited (10–13). This review aims to cover the molecular pathways involved in thyroid cancer progression, with an emphasis on anoikis resistance, investigating its role in metastasis and its potential as a therapeutic target.

Pathophysiology and Molecular Mechanisms of Thyroid Cancer

The pathogenesis of TC is a complex process influenced by genetic and environmental factors, including iodine deficiency or excess, exposure to ionizing radiation, and chronic inflammatory conditions (14). The development of TC involves numerous molecular genetic and epigenetic alterations that interfere with fundamental biological functions such as cell division, proliferation, and apoptosis regulation (2,7). At the molecular level, several genetic changes are observed during TC progression, including somatic mutations, gene amplifications, copy number alterations, and chromosomal rearrangements. These changes are regarded as traditional tumorigenic triggers that drive uncontrolled cancer cell growth and dissemination (7,14). Additionally, TC exhibits intratumoral heterogeneity, characterized by the presence of genetically, phenotypically, and behaviorally diverse cell populations within a single tumor. This heterogeneity arises from genetic and epigenetic factors among tumor subclones, such as copy number changes, point mutations, gene rearrangements, and epigenetic modifications. Genomic instability and the progressive accumulation of genetic alterations further contribute to the development of new subclones, increasing tumor complexity and promoting the transition from early-stage tumors to more aggressive cancers (15).

Epigenetic mechanisms, such as DNA methylation, play an important role in TC. DNA methylation can suppress gene expression, thereby altering cellular behavior and contributing to tumor development. The interplay between genetic alterations and epigenetic modifications underscores the complexity of tumorigenesis (7). In recent years, non-coding RNAs (ncRNAs) have also been identified as important molecules that play a regulatory role in TC. ncRNAs are involved in the post-transcriptional regulation of gene expression and imbalances in

these molecules may affect cancer pathogenesis. In particular, microRNAs (miRNAs) may contribute to cancer progression by modulating oncogenes or tumor suppressor genes in TC (2,16).

Genetic Alterations in Thyroid Cancer

Over the past three decades, advancements in genome sequencing technologies have facilitated a deeper understanding of the molecular mechanisms underlying TC. Although TC was considered a disease with a relatively low somatic mutation burden, driver mutations triggering cancer development have been identified in more than 90% of cases (7). The most frequently observed genetic alterations are point mutations in the *BRAF* and *RAS* genes, which are often followed by *RET/PTC* rearrangements. Less common mutations include alterations in *PIK3CA, TP53, TSHR, PTEN, GNAS,* and *CTNNB1* genes, all of which contribute to tumorigenesis (14). Notably, oncogenic gene fusions are much more prevalent in pediatric TC, present in 50–60% of cases, compared to only 15% in adults. Conversely, point mutations are less frequent in pediatric cases, observed in approximately 30% of pediatric TCs, compared with nearly 70% in adults (4).

The *BRAF* gene belongs to the serine-threonine kinase family and regulates the expression of genes involved in cell proliferation, differentiation, and apoptosis through the MAPK/ERK signaling pathway. The most frequently reported mutation is *BRAF-V600E*, which is associated with increased invasion, tumor growth, and loss of cell differentiation, particularly in PTC (2,4,7). BRAF mutations are found in up to 60% of adult PTC cases but are significantly rarer in pediatric PTC, appearing in only about 25% of cases, indicating age-related differences in the molecular drivers of TCs (3).

RAS gene mutations are among the most important genetic alterations following *BRAF* mutations (17). RAS functions as a G-protein that is inactive when bound to GDP and becomes active upon binding to GTP. There are three main isoforms of the *RAS* gene: *HRAS*, *KRAS,* and *NRAS*. Mutations in the *NRAS* gene, which primarily affect the PI3K/AKT pathway, are frequently implicated in thyroid tumorigenesis. However, these mutations also activate both the MAPK/ERK and PI3K/AKT signaling pathways. While *RAS* mutations are predominantly observed in FTCs, they have also been identified in other TC types, including PTC and poorly differentiated thyroid carcinoma (14). The frequency of *RAS* mutations varies between subtypes, occurring in 10–20% of PTC cases and 40–50% of FTC cases. However, *RAS* mutations are notably rare in pediatric TCs (18).

Another critical genetic alteration that plays a role in the pathogenesis of TC is the rearrangement of the *RET* proto-oncogene. The *RET* gene is expressed in neuroendocrine C cells and its mutations are primarily associated with MTC (7). However, *RET/PTC* rearrangements have been implicated in PTC carcinogenesis by leading to uncontrolled activation of MAPK/ERK and PI3K-AKT pathways (17). Notably, the prevalence of *RET/PTC* rearrangements differs between adult and pediatric cases, occurring in 5–15% of adult sporadic PTCs but rising to 25–65% in pediatric cases (19).

PAX8/PPARγ fusion protein is one of the most common genetic alterations in PTC after *RAS* mutations and has been associated with invasive tumors occurring at a younger age. This fusion protein has also been detected in follicular variant PTC, follicular adenoma, and Hurthle cell carcinoma (20). Additionally, recent studies have highlighted the higher prevalence of telomerase reverse transcriptase (TERT) promoter mutations in aggressive and undifferentiated tumors. These mutations, observed across all histological subtypes of TC, underscore their critical role in TC progression (Fig 1) (7,21).

Meanwhile, most cases of MTC are caused by mutations in the *RET* proto-oncogene. These mutations can occur as inherited germline mutations in MEN2A and MEN2B syndromes or arise sporadically. Mutations in *HRAS, KRAS,* and *NRAS* are responsible for a smaller number of sporadic MTC cases (7,22).

Signaling Pathways in Thyroid Cancer

Disruptions in the MAPK/ERK and PI3K/AKT signaling pathways play a critical role in the molecular pathogenesis of thyroid cancer (7). The MAPK/ERK pathway, one of the key signaling cascades involved in TC development, regulates cell proliferation, survival, and tumor progression. Genetic alterations, including mutations in *ALK, NTRK1, NTRK3,* and *EIF1AX*, which activate the MAPK/ERK pathway, have been discovered in cases where *BRAF* and *RAS* mutations are absent. This pathway is particularly significant in the development of PTC (22). *BRAF-V600E* mutation activates the MAPK/ERK signaling pathway and interacts with extracellular matrix (ECM) proteins in the thyroid tumor microenvironment and affects the viability, motility, and adhesion properties of cancer cells. This process is regulated through paracrine and autocrine signaling mechanisms between cancer cells and stromal cells, with these interactions in the tumor microenvironment leading to cancer progression (2).

PI3K/AKT pathway, another critical signaling cascade, plays a pivotal role in the development of FTC. It is activated by mutations in *PIK3CA* and *AKT1* genes or inactivation of the *PTEN* gene, in addition to *RAS* mutations and *PAX8/PPARγ* fusion protein (16). Furthermore, the PI3K/AKT pathway contributes to TC pathogenesis by influencing secondary molecular pathways, including WNT-β-catenin, Hypoxia Inducing Factor 1-α (HIF1α), FOXO3 and Nuclear Factor Kappa B (NF-κB) (2).

The progression of TC and its transformation into poorly differentiated TC or ATC is driven by additional mutations affecting key signaling pathways. Notably, disruptions in p53 and Wnt/β-catenin signaling pathways play an important role in this transformation. The NF-κB signaling pathway also contributes by regulating proliferative and anti-apoptotic mechanisms in TC cells, enhancing tumor survival and malignancy (7).

Thyroid Cancer and Anoikis Relationship

Anoikis: Cellular Mechanisms and Its Role in Cancer

Anoikis is a form of programmed cell death triggered by the detachment of cells from the ECM. This term, which means 'homelessness' in Greek, refers to the inability of cells to survive if they lose their connection with the ECM. Integrin receptors, which mediate cell-ECM interactions, not only establish a physical connection to the cytoskeleton but also regulate various cellular processes, including cell migration, proliferation, and survival, by transmitting signals from the ECM to the cell (23,24).

Anoikis serves as a crucial defense for the organism by preventing the reattachment and uncontrolled growth of misplaced cells within the ECM, thus maintaining tissue homeostasis (25). When anoikis is dysregulated, cells may survive or proliferate in inappropriate environmets, a hallmark of cancer cells that facilitates metastases to distant organs (26).

Intrinsic and Extrinsic Pathways of Anoikis

Anoikis is a process initiated by the activation of caspases and leads to the activation of endonucleases, DNA fragmentation, and cell death. This process is orchestrated through the two main pathways of apoptosis, intrinsic, and extrinsic pathways (Fig 2). Bcl-2 family proteins play a central role in both pathways and this family is divided into three main groups consisting of anti-apoptotic proteins such as Bcl-2, Bcl-XL, Mcl-1, pro-apoptotic proteins such as Bax, Bak and Bok, and pro-apoptotic proteins with BH3-only proteins such as Bid, Bad, Bim, Bmf, Noxa, Puma (23).

The intrinsic pathway is activated by intracellular stress factors, such as DNA damage or endoplasmic reticulum stress, leading to mitochondrial dysfunction. During this process, pro-apoptotic Bax and Bak proteins are transported from the cytosol to the outer mitochondrial membrane, where they oligomerize and form pores that increase mitochondrial permeability. This leads to the release of cytochrome-C, which combines with apoptosis protease activation factor (Apaf) and caspase-9 to form a complex called apoptosome. The apoptosome initiates apoptosis by activating caspase-3 (23,27). BH3-domain proteins, particularly Bid and Bim, play a crucial role in the intrinsic pathway of the anoikis. They are rapidly activated when cells detach from the ECM, facilitating the oligomerization of Bax and Bak, which in turn promotes the release of cytochrome C from the outer mitochondrial membrane. Furthermore, other BH3-only proteins, such as Bad, Bik, Bmf, Noxa, Puma, and Hrk indirectly promote Bax-Bak oligomerization by inhibiting the anti-apoptotic effects of Bcl-2 (28).

The extrinsic pathway is initiated by ligand binding to death receptors belonging to the tumor necrosis factor (TNF) receptor superfamily, such as Fas, TNFR1, and TRAIL receptor-1 and -2. The death-inducing signaling complex (DISC) is formed as a result of this binding. Caspases 8 are activated as a result of DISC's interactions with adaptor proteins, such as Fas-associated death domain protein (FADD). Once released into the cytoplasm, active caspase-8 activates effector caspases such as caspase-3, caspase-6, and caspase-7, leading to cell death (23,29). Furthermore, the Bid protein is cleaved and activated by caspase-8, which starts the release of mitochondrial cytochrome-C and apoptosome formation. This mechanism establishes a functional link between extrinsic and intrinsic pathways (30). Detachment of cells from the ECM increases the expression of Fas and Fas ligands.These changes reveal the important role of the extrinsic pathway in anoikis (31). Furthermore, alteration of cell shape may also lead to activation of Fas receptors and initiation of extrinsic anoikis (32). Anti-apoptotic signals contributing to anoikis resistance are summarized in Table 1.

Protection Against Anoikis Under Physiological Conditions

Epithelial cells are protected against anoikis when they bind to ECM proteins under physiological conditions. Studies have revealed that integrins suppress apoptosis and transmit cell survival signals through cell-ECM interactions (34). Various integrin subunits (α5β1, αvβ3, α1β1, α6β1) activate different signaling pathways that promote cell survival. These pathways include key signaling cascades such as focal adhesion kinase (FAK), Src kinase, integrin-linked kinase (ILK), PI3K/AKT, and MAPK/ERK. FAK, which is rapidly activated after contact with ECM, regulates cell survival, proliferation, and motility. PI3K/AKT pathway prevents cell death by inhibiting the activation of caspases and phosphorylating pro-apoptotic proteins (23). Additionally, ligand-independent activation of several growth factor receptors, including vascular endothelial growth factor receptor (VEGFR), hepatocyte growth factor receptor (HGF), platelet-derived growth factor receptor (PDGFR), and epidermal growth factor receptor (EGFR), can be triggered by ECM interaction. This activation activates the MAPK/ERK and PI3K/AKT pathways and transduces signals that inhibit cell death (23,35).

During cell migration, cells may temporarily lose their attachment to the ECM. Mesenchymal motility is the process by which cells migrate using specific adhesion points, driven by proteolysis of the ECM. During this type of migration, cell-ECM interactions and activation of growth factor receptors transduce survival-promoting signals by triggering the PI3K pathway (36). Another form of migration, ameboid motility, allows cells to move rapidly without proteolysing the ECM and without forming adhesion points. This process occurs by supporting cells that make poor contact with the ECM by transmitting survival signals through Rho family GTPases (23).

Cell-cell contacts play a crucial role in cell survival. Cadherins act as fundamental membrane proteins that provide binding between cells and interact with integrins and growth factor receptors to transmit survival signals to the cell. E-cadherin blockade triggers anoikis, while β-catenin overexpression increases anoikis resistance in epithelial cells. N-cadherin transduces survival signals via the PI3K/AKT pathway (38). Other cell surface molecules such as P- and L-selectin are also involved in this process, activating the FAK, Src, PI3K/AKT, and MAPK/ERK pathways, thus ensuring cell survival (39,40). Inhibition of the PI3K/AKT pathway, which transduces survival signals, impairs energy metabolism and decreases ATP levels in cells that are separated from the ECM. This may lead to an increase in reactive oxygen species (ROS) and trigger cell death. However, autophagy comes into play as a temporary survival mechanism under these conditions. During autophagy, cells generate energy by breaking down their components and can survive when they reattach to the ECM (41).

Anoikis Resistance in Cancer Cells

In normal cells, anoikis functions as a vital mechanism for elimination of abnormal cells and maintenance of tissue homeostasis. In contrast, cancer cells can develop resistance to anoikis, enabling them to evade apoptosis when detached from the ECM. This resistance plays a critical role in cancer progression and metastatic spread. By avoiding anoikis through various molecular and cellular reprogramming processes, cancer cells can survive at the primary tumor site and metastasize to distant organs. These resistance mechanisms involve several key processes such as changes in integrin profile, epithelial-mesenchymal transition (EMT), constitutive activation of survival signaling pathways, and metabolic adaptations, including Warburg effect and autophagy (23,42).

One of the most important mechanisms enabling cancer cells to survive despite detachment from the ECM is changes in the expression profile of integrins. These changes allow cancer cells to acquire resistance to anoikis and survive both in the early stages of oncogenic transformation and during metastatic colonization. For instance, the overexpression of integrin subunits such as αvβ3 and α5β1 leads to activation of signaling pathways that promote cancer cell survival. Molecules like FAK and Src kinase are activated through integrins, triggering PI3K/AKT and MAPK/ERK signaling pathways (Fig 3). These pathways transmit signals that promote cell survival, inhibit caspase activation, and lead to inactivation of apoptotic proteins. Furthermore, these signals facilitate metastatic spread by increasing the motility of cancer cells (23,42,43).

Epithelial-mesenchymal transition (EMT) is another physiological process characterized by epithelial cells reorganizing their cytoskeleton, losing connections, and acquiring a motile phenotype. In cancer cells, EMT has been shown to enable detachment from neighboring cells, resistance toanoikis, migration to distant tissues, and invasion. During EMT, the expression of cell-cell adhesion molecules like E-cadherin is suppressed, while mesenchymal markers such as vimentin, fibronectin, and N-cadherin are activated, reducing ECM dependence and enabling metastasis (44). EMT-inducing transcription factors (Snail, Twist, NF-κB, ZEB1/2) activate the PI3K/AKT pathway in cancer cells, suppress pro-apoptotic proteins, and protect against cell death (Fig 3) (33). Hypoxia-inducible factors (HIFs), particularly HIF-1, trigger EMT in hypoxic tumor microenvironments by increasing Twist and NF-κB activation and promoting Snail expression. Furthermore, HIF-1 activates the MAPK/ERK pathway, leading to the degradation of pro-apoptotic proteins and increasing anoikis resistance (45).

Resistance to anoikis in cancer cells is closely associated with the reprogramming of apoptotic mechanisms despite the loss of integrin signaling. These cells evade apoptosis by overexpression of anti-apoptotic proteins or suppression of pro-apoptotic proteins. The PI3K/AKT pathway, a critical survival signaling cascade, is activated via integrins and growth factor receptors, ensuring cell survival (33,46). Resistance to anoikis is further enhanced when negative regulators of this pathway, such as PTEN, are inactivated (23,33). In addition, Src family kinases and ILK strengthen resistance to anoikis, promoting ECM-independent survival, invasion, and metastasis. (47).

The regulation of anoikis resistance in cancer cells due to oxidative stress and hypoxia plays a critical role in tumor survival, proliferation, and metastatic spread. Chronic ROS production enhances survival signaling and suppresses apoptotic pathways by activating enzymes like NADPH oxidase, leading to sustained PI3K/AKT and MAPK/ERK pathway activity (Fig 3). Redox-sensitive transcription factors such as NF-κB, HIF-1α, and p53 also contribute to anoikis resistance (48). The continuous generation of ROS leads to the inhibition of enzymes involved in apoptotic processes, such as PTEN and PTP-1B, through oxidative modification of phosphatases. Increased ROS levels also activate antioxidant responses, enabling cancer cells to persist even without ECM attachment. (23,41).

Cancer cells reprogram their metabolic pathways to sustain survival under ECM-deprived conditions. Unlike normal cells, which rely on oxidative phosphorylation for energy production, cancer cells shift their metabolism to glycolysis, a process known as the Warburg effect. Upon detachment from the ECM, glucose uptake, glycolysis, and mitochondrial respiration typically decrease, leading to ATP depletion and energy deficiency. However, cancer cells compensate by maintaining energy production through glycolysis, achieved by increasing glucose uptake and activating signaling pathways such as PI3K/AKT and HIF-1α (23,42). In addition, cancer cells activate the autophagy pathway to manage metabolic stress. During autophagy, these cells maintain energy balance and suppress apoptotic signaling. By integrating metabolic reprogramming and autophagy, cancer cells effectively adapt to hostile environments, ensuring survival and facilitating metastatic progression (42).

Cancer progression to malignancy consists of a series of steps, including carcinogenesis, sustained proliferative signaling, the formation of a hypoxic tumor environment, angiogenesis, lymphangiogenesis, interaction with tumor microenvironment, ECM migration and invasion, intravasation into the bloodstream, survival in circulation, extravasation, metastatic niche preparation, and proliferation in distant organs

(49). Resistance to anoikis plays a critical role in many of these processes. Therefore therapeutic approaches targeting anoikis resistance hold important potential for halting cancer progression. (23).

Role of Anoikis in Thyroid Cancer

TC subtypes exhibit significant differences in biological behavior and therapeutic response. PTC is the most common subtype and is typically associated with a favorable prognosis (1). However, 15-50% of PTCs exhibit local invasion and regional lymph node metastases. In contrast, FTC is more invasive and tends to metastasize to distant organs with a rate of 20%. ATC is the most aggressive subtype and exhibit metastases to distant sites in over 98% of cases. These differences in metastatic characteristics make TC a valuable model for exploring the underlying mechanisms of metastasis (1,10).

Investigation of cell death processes is crucial for understanding the molecular mechanisms driving metastasis in TC. Among these processes, apoptosis is the most extensively studied (50–52). According to existing research, one of the most prevalent molecular changes in TC is the activation of the PI3K/AKT signaling pathway, which promotes cancer cell survival by triggering anti-apoptotic signals. Furthermore, overexpression of Bcl-2 family proteins plays an important role in the suppression of apoptotic processes (50). Specifically, mutations in the p53 tumor suppressor gene, which are prevalent in ATC, affect the operation of apoptotic processes, which in turn leads to a more aggressive phenotype of cancer cells (53).

Anoikis resistance is essential for TC cells to detach from the ECM and survive during the metastatic process. Although studies on anoikis resistance in TC are limited, it is recognized as a critical factor in tumor progression and metastasis (10–12,54). Research has shown that TC cells increase their invasive capabilities through EMT. Metastatic TCs are characterized by overexpression of mesenchymal markers such as osteopontin and vimentin (55). Signaling pathways that directly regulate EMT include integrin, Notch, TGF-β, NF-κB, and PI3K. Some of these pathways have been found to play an important role in PTC progression (56). Furthermore, the activation of the PI3K/AKT signaling pathway plays a critical role in TC progression. Nuclear AKT activation has been identified in TC cells invading surrounding tissues, underscoring its importance in facilitating metastasis (57).

Although studies on the mechanisms by which TC cells develop resistance to anoikis are limited, this area has garnered significant research interest. Recent findings highlight various molecular pathways and cellular interactions that contribute to anoikis resistance in TC. A study revealed that TC cells detached from the ECM communicate with each other through gap junctions to transmit anti-apoptotic signals. In this process, the overexpression of the Connexin 43 gap junction protein enhances the survival capacity of cancer cells during metastasis. In particular, sustained activation of the AKT signaling pathway in cells carrying the *BRAFV600E* mutation has been shown to develop resistance to anoikis. Furthermore, inhibition of gap junction-mediated intercellular communication (GJIC) suppressed AKT activation and led to apoptosis in cells with high PTEN expression. These findings suggest that GJIC could serve as a potential therapeutic target in the treatment of TC (54). In another study, the effects of integrin β4 on PTK were investigated and it was found that β4 expression was significantly increased in PTC compared to normal thyroid tissues. This upregulation was associated with extrathyroidal extension, lymph node metastasis, and higher tumor stage. Additionally, it has been highlighted that integrin β4 enhances the capacity of circulating tumor cells to metastasize, and this impact is linked to the activation of the PI3K, MAPK/ERK, and NF-κB signaling pathways. Additionally, prior *in vitro* studies have demonstrated that tumor cells' resistance to invasion, migration, and anoikis is enhanced when β4 expression is upregulated, whereas these functions are diminished when β4 is suppressed (58,59).

Zhao et al. investigated the role of cell cycle checkpoint kinase 2 (CHK2) protein on tumor progression and metastasis in PTC cells. CHK2 is a tumor suppressor kinase that maintains genomic integrity in response to DNA damage. The study revealed that CHK2 levels were high in PTC tissues but significantly reduced in lymph node metastases. These findings suggest that CHK2 is associated with tumor aggressiveness, particularly lymph node metastasis. Moreover, CHK2 was found to suppress anoikis resistance through the PRAS40 signaling pathway. PRAS40 is an AKT kinase-dependent substrate that promotes cell growth and survival by regulating mTORC1 activity. It was observed that suppression of CHK2 prevented anoikis by increasing p-PRAS40 levels in a p53-independent manner. In conclusion, CHK2 regulates the anoikis via PRAS40, presenting a potential therapeutic target for cancer treatment (11). Yi Pan et al. found that anoikis resistance in PTC could be inhibited by miR-363-3p targeting integrin α6. This study highlights the role of miR-363-3p in the metastatic process and its clinical potential in preventing metastasis (60). These findings supported earlier research demonstrating the dual functions of miRNAs as pro- and anti-metastatic agents in metastatic processes, including invasion, cell migration, anoikis resistance, and EMT (61,62).

A study investigating anoikis-related genes in patients with TC identified six critical genes such as *EZH2, PRKCQ, CD36, INHBB, TDGF1,* and *MMP9*. Functional analyses revealed their significant roles in tumor progression, cell migration, and cancer-related signaling pathways. It has been found that *EZH2* enhances cell motility by weakening the connection of cells to the ECM, thereby promoting anoikis resistance; *PRKCQ* strengthens anoikis resistance by activating the MAPK/ERK pathway through its kinase activity; and blocking *CD36* eliminates anoikis resistance by reducing integrin sequestration. *INHBB* has been shown to inhibit cell migration by suppressing anoikis resistance through the TGF-β signaling pathway; TDGF1 has been shown to increase both anoikis resistance and invasion potential and MMP9 has been shown to trigger anoikis by degrading intercellular binding components and acting on the epithelium. The contribution of these genes to anoikis resistance was elucidated in detail and ROC curves confirmed that these six genes are a strong indicator for prognosis prediction in patients with PTC. According to the risk scores, patients were divided into high and low-risk groups; immunological analyses revealed that plasma cells, T cells, and macrophages were more infiltrated in the high-risk group, whereas CD8+ T cells and NK cells were found at lower levels. These findings support the critical role of the immune microenvironment in tumor development and its relationship with anoikis resistance (63). On the other hand, a proteomic study by Saharat et al. aimed to identify the key proteins involved in anoikis resistance in TC cell lines and found that overexpression of tumor susceptibility gene 101 (TSG101) protein plays a critical role in TC cells with high metastatic potential. It has been suggested that TSG101 is involved in cellular processes such as vesicle biogenesis, endocytosis, membrane transport, and receptor recycling. Its overexpression may contribute to the retention of death receptors via endocytosis, leading to anoikis resistance (10). TSG101 has been implicated as an oncogenic factor in various cancers, including breast, prostate, and hepatocellular carcinoma. Suppression of TSG101 has been shown to arrest the cell cycle in these cancers, suggesting its potential as a therapeutic target (64–66). These findings reveal that TSG101 plays a pivotal role in regulating anoikis resistance in TC and may serve as a molecular therapeutic target against metastatic TC (10).

In conclusion, these studies highlight that anoikis resistance plays a critical role in tumor progression and metastasis in TC. Molecules such as TSG101, CHK2, integrin β4, and miR-363-3p have emerged as potential therapeutic targets, especially in metastatic TC. Additionally, genes such as *EZH2, PRKCQ, CD36, INHBB, TDGF1,* and *MMP9* have been demonstrated to play important roles in anoikis resistance and tumor progression. These findings offer potential advancements in the treatment of metastatic TC by providing a promising foundation for the development of new strategies targeting anoikis resistance.

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Table 1. Overview of anti-apoptotic mechanisms contributing to anoikis resistance in thyroid cancer.

Mechanism / Pathway	Key Molecules	Mechanism of Action in Anoikis Resistance
Intrinsic apoptotic pathway inhibition	Bcl-2, Bcl-XL, Mcl-1	Prevents mitochondrial cytochrome-C release and caspase-9 activation.
Extrinsic apoptotic pathway suppression	Fas, FasL, FADD, Caspase-8	Inhibits caspase-8-mediated apoptosis and maintains Bid in an inactive form.
PI3K/AKT pathway activation	PI3K, AKT, PTEN (loss)	Suppresses pro-apoptotic proteins and promotes survival under ECM-detached conditions.
MAPK/ERK pathway activation	BRAF (V600E), MEK, ERK	Enhances cell proliferation and survival, suppresses pro-apoptotic signaling, and supports EMT induction.
NF-κB pathway activation	NF-κB	Induces transcription of anti-apoptotic genes (e.g., Bcl-2).
Integrin-mediated survival signaling	αvβ3, α5β1, FAK, Src, ILK	Maintains survival via ECM-independent activation of AKT/MAPK signaling.
Growth factor receptor activation	VEGFR, HGF-R, PDGFR, EGFR	Activates PI3K/AKT and MAPK/ERK pathways via ligand-independent signaling, enhancing survival.
Hypoxia response and ROS adaptation	HIF-1α, NADPH oxidase, ROS, NF-κB, p53	Promotes EMT and survival under oxidative stress, suppresses apoptosis via redox modulation.
EMT	Snail, Twist, ZEB1/2, N-cadherin, Vimentin	Suppresses E-cadherin, promotes mesenchymal traits, enhances motility, invasion, and anoikis resistance.
Cadherin switching and β-catenin signaling	E-cadherin (downregulated), N-cadherin (upregulated), β-catenin	Reduces intercellular adhesion and promotes survival during ECM detachment.
Metabolic reprogramming (Warburg effect)	GLUT1, LDHA, HIF-1α	Maintains ATP levels by increasing glycolysis during ECM detachment.
Autophagy activation	Beclin-1	Provides temporary survival through metabolic stress adaptation.

Abbreviations: ECM, extracellular matrix; FAK, focal adhesion kinase; Src, Src family kinases; ILK, integrin-linked kinase; PI3K, phosphoinositide 3-kinase; AKT, protein kinase B; NF-κB, nuclear factor kappa B; VEGFR, vascular endothelial growth factor receptor; HGF-R, hepatocyte growth factor receptor; PDGFR, platelet-derived growth factor receptor; EGFR, epidermal growth factor receptor; EMT, epithelial-mesenchymal transition; ROS, reactive oxygen species; GLUT1, glucose transporter 1; LDHA, lactate dehydrogenase A; HIF-1α, hypoxia-inducible factor 1-alpha.

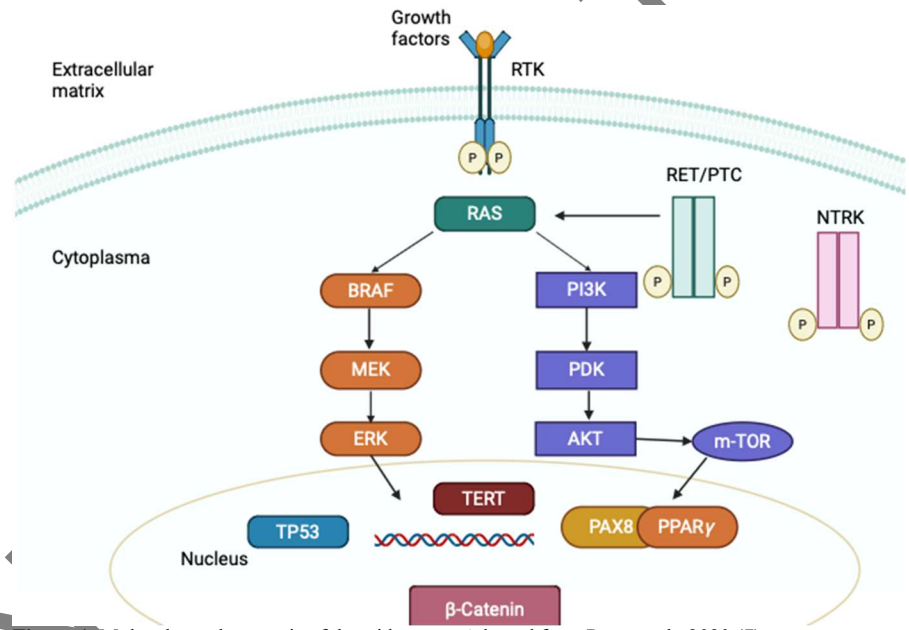

Figure 1. Molecular pathogenesis of thyroid cancer. Adapted from Prete et al., 2020 (7).

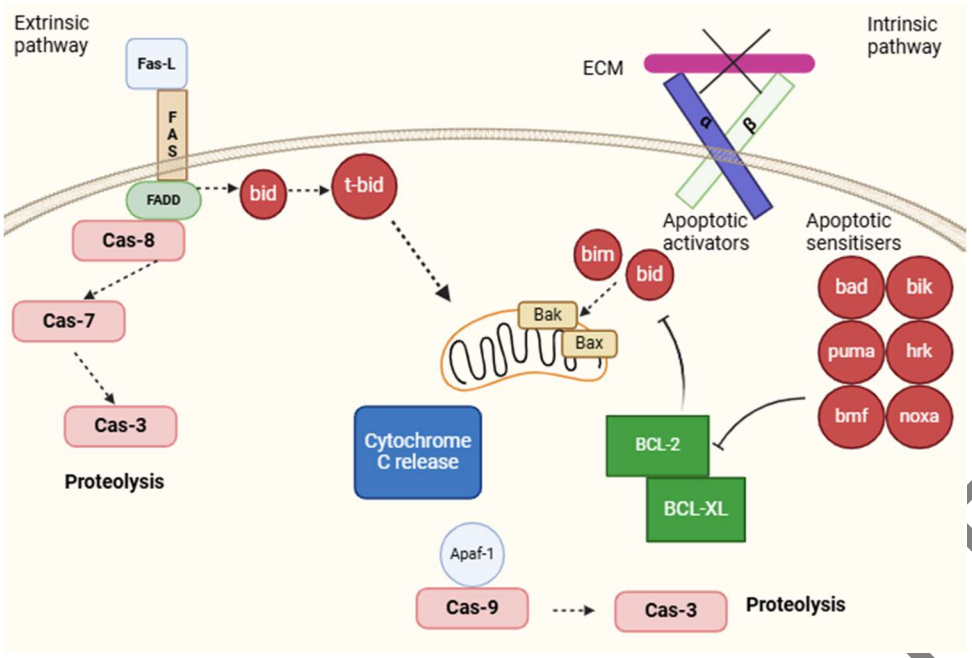

Figure 2. Activation of anoikis mechanism. Adapted from Taddei et al., 2012 (33).

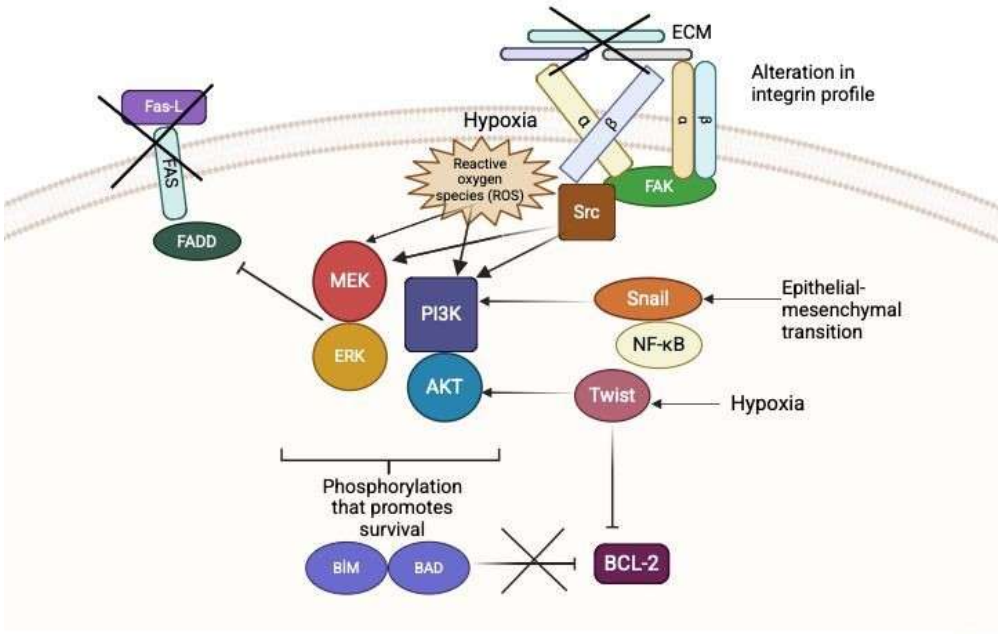

Figure 3. Molecular mechanisms of anoikis resistance in cancer cells. Adapted from Taddei et al., 2012 (33).