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Molecular mechanisms in differentiated thyroid cancer

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1. ABSTRACT

Thyroid cancer is a common endocrine malignancy. The tumorigenesis of thyroid tumours has been identified in recent years, including numerous genetic alterations and several major signalling pathways. However, the molecular mechanisms involved in thyroid cancer metastasis remain controversial. Studies in thyroid cancer metastasis suggested that reactivation of several pathways, including epithelial to mesenchymal transition and microenvironment change, may be involved in thyroid cancer migration. The previously identified thyroid oncogenes, BRAF, RET/PTC and Ras, play important roles in regulating the metastatic process. Here, we review the recent knowledge eon molecular mechanisms involved in thyroid cancer metastasis.

2. INTRODUCTION

Thyroid cancer is a common endocrine malignancy with an increasing incidence in the past decades worldwide (1). Many countries have two-fold increase incidence of thyroid tumor since the late 1990s. In some regions, like Hong Kong, New Zealand, and UK, thyroid cancer has the most progressive prevalence (1). In China, the Ministry of Health of China reported thatthyroid cancer was the third most malignant tumor in female in 2012. The cases of thyroid cancer increased by 225.2% in the last nine years in Beijing (2).

The histological types of thyroid cancer are papillary thyroid cancer (PTC), follicular thyroid cancer (FTC), poorly differentiated thyroid cancer (PDTC) and anaplastic thyroid cancer (ATC). PTC and FTC are collectively classified as differentiated thyroid cancer (DTC). Parafollicular C cell-derived medullary thyroid cancer (MTC) counts a small proportion of thyroid malignancies.

The majority of DTC patients have better prognosis. However, patients with extensive local ordistant metastasis frequently fail to respond to the standard treatments and tend to have a worse prognosis with a 50% five-year survival rate (3,4). Metastasis is the most common cause of death in thyroid cancer. Bone metastasis often predicts a significantly worse prognosis (5). Distant metastatic disease is present in only 3–15% of patients with thyroid cancer and 6-20% of patients develop metastasis (6). Some study reported that lymph node metastasis has little relation with survival, but it may cause serious complications and recurrence. Generally, follicular thyroid cancer spreads via the blood stream, whereas papillary thyroid tumor spreads via the lymphatic system (7).

To identify the mechanisms of metastasisin thyroid cancer may throw light ondevelopingtherapeutic targets for patients with progressive metastatic disease.

3. ONCOGENES, METASTASIS SUPPRESSORSAND MICRORNAS: COMMON GENETIC ALTERATIONS IN THYROID CANCER

Numerous genetic alterations that playa fundamental role in the tumorigenesis of thyroid tumours have been reported,such as BRAF, RET/PTC, RAS, TRK in papillary thyroid cancer and RAS, PTEN, and PAX8/PPAR gamma mutations in follicular thyroid cancers (8-10), However, the molecular mechanisms involved in thyroid cancer metastasis remain unclear.

3.1. Oncogenes

Various oncogenes expressed in thyroid cancer are potentially associated with invasion and metastasis. Oncogenes can induce chromosomal instability and epithelial-mesenchymal transition (EMT), activate pathways that lead to degradation of local intracellular matrix proteins, and also induce recruitment of bone marrow progenitor cells that may facilitate angiogenesis (11).

The $BRAF^{T1799A}$ (V600E) mutation is the most common oncogenic event identified in PTC (12). PTCs with$BRAF^{T1799A}$ (V600E) are often invasive and tend to proceed to an advanced stage (13). Kim *et al* (14) reported that 76% of patients with a BRAF mutation and PTC had lymph node metastasis. As noted above, BRAFT1799A (V600E) could be associated with local invasion and nodal metastases.

RAS mutations rank the second in the prevalence to BRAF mutations in thyroid cancer (15). RAS mutations could be markers for aggressive cancer and RAS genotyping can identify thyroid cancer subsets together with prognosis (16). RAS expression can also be associated with aggressiveness and poor prognosis in thyroid cancer (17). Although RAS is a classical dual activator of MAPK and PI3K-AKT pathways, RAS mutations preferentially activate the PI3K–AKT pathway where AKT is phosphorylated in thyroid cancers (18,19).

Rearrangement of the RET gene, also known as RET/PTC rearrangement, is the most common genetic alteration identified in thyroid papillary cancer. RET/PTC is more commonly seen in children and young adults. RET/PTC in papillary cancer is associated with radiation exposure (20). PTCs harboring the RET/PTC3rearrangement demonstrates a high metastatic potential (21). RET/PTC is a classical oncoprotein that activates the MAPK and PI3K–AKT pathways (22,23).

Another oncogene, c-Met, has been found to play critical roles in neoplastic diseases (24). c-Met expression may correlate with poor prognosis of PTC, lymph node metastasis and pathological stage (25). The paired box 8 (PAX8)–peroxisome proliferator activated receptor-γ (PPARG) fusion gene (PAX8–PPARG) is another prominent recombinant oncogene in thyroid cancer, occurring in up to 60% of FTC (26-28) with indications of invasion and poor prognosis.

3.2. Metastasis suppressors

Metastasis suppressor genes encode proteins that inhibit metastasis without altering malignant transformation (29,30). Studies showed that the expression of metastasis suppressors were reduced in metastatic tumour cells, compared with tumorigenic but non-metastatic tumour cells (30). More than twenty metastatic suppressor genes have been identified (31). Revealing the mechanism of how metastatic suppressors are delivered may provide potential therapeutic targets.

A number of genes that encode metastasis-suppressing transcripts have been identified, including NM23, CAD1, MKK4, KAI-1 (CD82), TXNP, CRSP3, BRMS1, KiSS-1, and etc. (29,30). Several of these genes have been studied in thyroid cancer, including NM23 (32,33), CAD1 (34,35), KAI-1, KiSS-1 (36), GPR54 (36,37), and RCAN1-4 (38). NM23, CAD1, and KAI-1 are downregulated in invasive and metastatic cancer.

KAI-1 is a prominent metastatic suppressor gene that was originally identified in prostate carcinoma and mapped to human chromosome 11p11.2 (17). KAI-1 is significantly downregulated in progressive papillary carcinoma, including lymphnode metastasis, and its anaplastic transformation (39).

Carles *et al.* (32) used monoclonal antibody to observe NM23-H1 in patients with follicular carcinoma. Results showed a significant inverse association between metastatic disease and the expression of NM23-H1 product. The NM23-H1 protein immunoreactivity was inversely associated with the metastatic potential of tumors and the mortality of patients with follicular thyroid carcinoma (32). Arai et *et al* (33,40) and Okubo*et al*. (41) reported that NM23-H1 was lower in metastatic lymph node tissue than in the primary tumor indifferentiated thyroid cancer.

Lee *et al*. described the product of KiSS-1 as an inhibitor of tumor metastases in human melanoma and breast carcinoma cell lines (42,43). Matthew *et al*. demonstrated that metastin, the KiSS-1 gene product receptor, is overexpressed in PTC, but is rarely expressed in FTC, as papapillary cancer are less likely to develop distant metastases than follicular cancers (36). The KiSS-1gene products have been identified as the endogenous ligands for a heptahelical G protein-coupled receptor (GPR54). The expression of GPR54 was maintained in primary PTC and was reduced in FTC, consistent with the greater tendency of FTC to metastasize hematogenously (36).

3.3. MicroRNA

Several studies analyzed the expression of microRNAs (miRNAs or miRs) in thyroid carcinoma andevaluated a possible role of the deregulation in the process of carcinogenesis (44). MicroRNAs (miRNAs or miRs) constitute a class of small endogenous noncoding RNAs of 19 - 23 nucleotides that negatively regulate gene expressions (45). MicroRNAsare an abundant class of gene regulatory molecules in multicellular organisms and modulate the expression of many protein-coding genes (45). Functioning as either oncogenes or tumor suppressors, miRNAs contribute to tumorigenesis. The collective studies have revealed that the most differentially expressed miRNAs in PTC, including miRNA-146b, -221, -187, -30d (46) and MiR-155 (47) are up-regulated. Mazeh *et al*. comparatively analyzed twenty-seven fine needle aspiration Biopsy (FNAB) samples from twenty PTC patients. The results showed that a 95% sensitivity of miRNA-221 in detecting PTC (48).

Eleven miRNAs were identified as putative markers of invasion and metastasis of PTC by transwell invasion experiments *in vitro*. The miRNA microarray technique was used to validate the differential expression of these eleven miRNAs between invasive cancer cell lines and their respective non-invasive controls (49). MiR-146b was significantly overexpressed in PTCs with extrathyroidal invasion and associated with high-risk PTC with BRAF mutation (50). MiR-146b expression isan independent risk factor for poor prognosis in PTC together with cervical lymph node metastasis.

4. PATHWAYS AND METASTASIS IN THYROID CANCER

4.1. The MAPK signalling pathway

Mitogen-activated protein kinases (MAPK) are well-conserved enzymes connecting cellsurface receptors to intracellular regulating targets. There are three well-known MAPK subfamilies: extracellular signal-regulated kinases (ERK), c-Jun NH2-terminal kinases (JNK), and p38 MAPK isoforms (51).

This pathway has been well studied in thyroid tumorigenesis and is very important in PTC (52,53). In thyroid cancer, the MAPK pathway is driven by activated mutations, including BRAF and RAS mutations by RET/PTC and ALK mutation s (54). The activation of BRAF-V600E-mediated MAPK pathway promotes the release of thrombospondin 1(TSP1) into the extracellular matrix (ECM), where TSP1interacts with and modulates other proteins, including integrins and non-integrin cell-membrane receptors, matrix proteins, cytokines, VEGFA and MMPs. In turn, these modulated proteins activate downstream signaling in thyroid cancer cells and promote tumour progression and metastasis (55,56). Studies have also demonstrated increased expression of MMP-2 in metastatic thyroid cancer (57). The expression of phosphorylated JNK (p-JNK) correlates with the aggressive clinicopathological features inPTC. Indeed, the presence of lymph node metastases and advanced TNM stages both positively correlated with the level of p-JNK (58).

4.2. The PI3K-AKT signalling pathway

Akt is a critical mediator of growth factor-activated PI3k signaling, which is central to the regulation of benign thyroid cell growth (59-62). The PI3K–AKT pathway has a fundamental role in thyroid tumorigenes is as a regulator of cell migration and a critical modulator of invasion in both human thyroid cancer and thyroid cancer cell lines (63).

Akt activation is associated with the pathogenesis of inherited thyroid cancer and in sporadic thyroid cancers (63). Human studies suggested that the invasiveness and metastasis of FTC were promoted by the PI3K–AKT pathway, particularly in the activation and nuclear localization of Akt 1(63). Nuclear translocations of Akt1 and p-Akt were associated with cell invasion and migration in human thyroid cancer cells (63), which correlate with the presence of Akt1mutations in metastatic thyroid cancers (64). The thyroid hormone receptor β PV/PV knock-in (PV) mice was developed to further study the metastasis in differentiated thyroid cancer *in vivo* and pathways involved in the metastatic progression *in vitro* (65). Saji *et al*. demonstrated that Akt1 ablation delayed tumor progression, vascular intravasation and distant metastasis inβPV/PV-Akt1 KO mice. Therefore, the MAPK pathway has a central role in PTC, while the PI3K–AKT pathway has a crucial role in the invasion and metastasis of FTC (15). Follicular thyroid cancer cells invading the tumour capsule or blood vessels, or other areas, were characterised by Akt activation in a nuclear pattern, suggesting an association of Aktactivity and tumor aggressiveness and metastasis.

4.3. The WNT-β-catenin signalling pathway

The expression of β-catenin was higher in ATC than in DTC. Thus, the WNT-β-catenin pathway is believed to have a primary role in thyroid tumour aggressiveness (66).The activation of PI3K-Akt pathway, where glycogen synthase kinase 3β (GSK3β) is directly phosphorylated and then inactivated by Akt, lead to the aberrant activation of WNT-β-catenin signaling pathway (67,68)

4.4. Other signalling pathways

nuclear factor-κB (NF-κB) activation is increased in thyroid cancer cell lines and tissues (69,70). HIF1α is expressed in thyroid cancers, particularly in aggressive types, such as ATC, promoting cancer progression (71,72). The oncogene MET, another target of HIF1α, is also over-expressed due to the upregulated HIF1α in thyroid cancer (72). Lymphatic metastases were highly positive (>93%) for both signal transducer

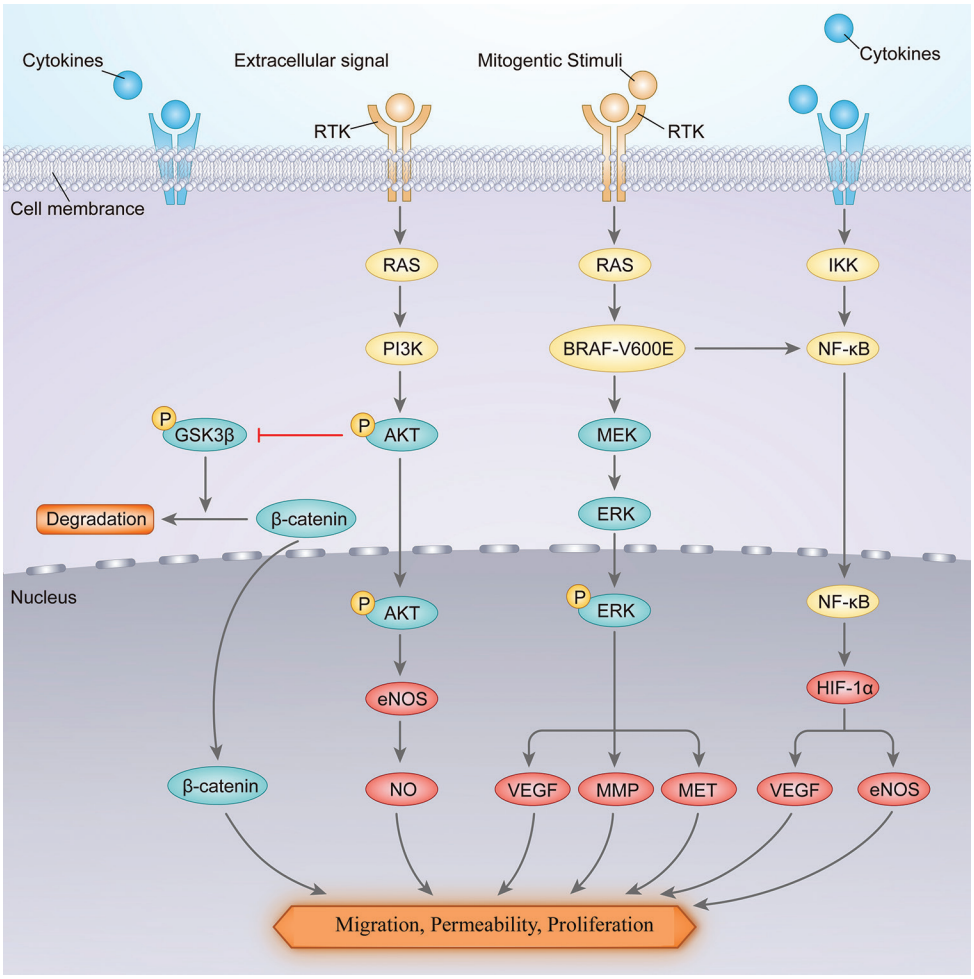

Figure 1. Main pathways involved in metastatic progression in thyroid cancer.The MAPK pathway is driven by activated mutations, including BRAF and RAS mutations, promotes matrix proteins, cytokines, VEGFA and MMPs.Extracellular signals activate receptor tyrosine kinases (RTKs) in the cell membrane, leading to theactivation of RAS and PI3K and consequently leads to phosphorylation (P) and activation of AKT. PhosphorylatedAKT inducestumour-promoting genes.The activation of PI3K-Akt pathway, where GSK3β is directly phosphorylated and then inactivated by Akt, lead to the aberrant activation of WNT-β-catenin signaling pathway.The NF-κB pathway, stimuliactivatedby receptors in the cell membrane, lead to downstream free NF-κB entering the nucleus to promote the expression of tumour-promoting genes.As a result, thease signalings are activated leading to migrationand cell proliferation.

and activator of transcription 3(STAT3) and p-STAT3. The STAT3 pathway is ubiquitous in PTC and p-STAT3 is significantly upregulated in metastatic PTC (73) (Figure 1).

5. EPITHELIAL–MESENCHYMAL TRANSITION AND THYROID CANCER METASTASIS

EMT was first recognized as a differentiation process in early embryogenic morphogenesis (74). It is a coordinated molecular and cellular process of reduction incell to cell adhesion, apical-basolateral polarity, epithelial markers, an acquisition of motility, spindle-cell shape, and mesenchymal markers (75). The inclusive EMT process indicates a potential mechanism that enhances the detachment of cancer cells from the primary tumors (75). Besides TGFβ and RTK/Ras signaling, autocrine factors and Wnt-, Notch-, Hedgehog- and NF-κB-dependent pathways were reported to contribute to EMT (11). Transforming growth factor-h (TGF-h), epidermal growth factor (EGF) family members, fibroblast growth factors (FGF), hepatocyte growth factor (HGF), and insulin-like growth factor (IGF) can induce EMT in an autocrine or paracrine manner (11). Furthermore, miR-200 plays a key role in EGF/EGFR-mediated thyroid cell invasion and in EMT *in vitro* (76).

E-cadherin, one of the caretakers of the epithelial phenotypes, is involved in EMT (77).The downregulation of E-cadherin was first reported more than a decade ago by Graff and his colleagues (78).Their results showed that the DNA methylation of the E-cadherin gene, CDH1, promoter varies at different stages in the metastatic process (79).

Recent studies suggested that EMT has an important role in thyroid cancer cell migration. E-cadherin expression could be associated with the de-differentiation, progression, and metastatic spread of thyroid carcinomas (34). The expression of E-cadherin is significantly lower in PTC with lymph node metastasis than in non-metastatic cases (36). Brabant *et al*. (78) concluded that both gene expression and post-transcriptional control of E-cadherin may be impaired in human thyroid cancers. Vimentin, a mesenchymal cell marker, is frequently over-expressed in metastatic PTCs (80).

Conclusively, the close crosstalk between oncogenes-activated signaling pathways and the EMT-related signaling pathways contribute to the aggressiveness and metastases of thyroid cancer.

6. MICROENVIRONMENT AND THYROID CANCER METASTASTIC

There is increasing notice in the stromal microenvironment, where the development of neoplastic cells influences various steps in cancer progression, including tumor cells metastasis and the regulation of malignant cell behavior (81). Although tumor cells are the driving force of metastasis, new findings suggested that the host cells within the tumor microenvironment also play a critical role in altering metastatic behavior (82).

The microenvironment is mediated largely through bidirectional interactions between epithelial tumor cells and neighboring stromal cells, such as and endothelial and immune cells (81). The interactions include adhesion, survival, proteolysis, migration, immune escape mechanisms lymph-/angiogenesis, and homing on target organs.

Lymphocyte infiltration commonly occurs in PTC, particularly those with RET/PTC mutations. Inflammationis associated with the development and prognosis of PTC (83,84). Recent report suggested that the specific types of infiltrating lymphocytes influence the tumor size and local metastatic spread (85).

Single cancer cells or small clusters of cancer cells may release small particles, including exosomes and microvesicles, to modifytissues to better accept cancer cells (82).Tumors secrete large, plasma membrane-derived microvesicles, which carry matrix metalloproteinases (86,87). Microvesiclescan help the migration of tumor cells within a solid tissue (88). Exosomesare known to carryproteins, lipids, and RNAs, mediate intercellular communication in different cell type, and function in both physiologicaland pathological conditions (88). Tumor derived exosomes can participate in metastatic dissemination of tumor cells by educating bone marrow progenitor cells and promoting their migration to thefuture sites of metastasis (89), by directly seeding tumor-draining lymph nodes before further migration of tumor cells themselves (90), or by increasing local motility of tumor cells via a complex impact with surrounding fibroblasts (91). Exosomes from cultured glioblastoma tumor cells contain several angiogenic peptides and RNAs that can be transferred and translated into recipient brain microvascular endothelial cells, respectively. Exosomes can also confer proangiogenic properties and then disseminatemalignancy (92). The role of exosomes in thyroid cancer metastasis needs to be further eludidated.

7. CONCLUSION

In differentiated thyroid cancer, even the presence of vascular invasion in small tumors predicts distant metastases. These metastatic lesions are often located in the lymph nodes, lungs or bones and are identified based on thyroglobulin elevations, which predictsa poor prognosis. Common genetic alterations, oncogenes, metastasis suppressors and microRNAs all play critical roles in the progression of thyroid cancer. Furthermore, pathways, including MAPK, PI3K-Akt, WNT-β-catenin and etc., all cooperate in the promotion of cancer metastases. More importantly, the recogniction of the role of EMT and microenvironment in the metastatic mechanism of thyroid cancer is rising. A firm understanding of how thyroid cancer cell progression is regulated in different metastatic mechanisms and environments will help develop effective therapeutic targets in progressive metastatic thyroid cancer.

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Abbreviations: PDTC: poorly differentiated thyroid cancer; PTC: papillary thyroid cancer; ATC: anaplastic thyroid cancer; MTC: medullary thyroid cancer; DTC: differentiated thyroid cancer; EMT: epithelial-mesenchymal transition; PAX8: paired box 8; FNAB: fine needle aspiration Biopsy; MAPK: Mitogen-activated protein kinases; ERK: extracellular signal-regulated kinases; JNK: c-Jun NH2-terminal kinases; ECM: extracellular matrix; TSP1: thrombospondin 1; GSK3β: glycogen synthase kinase 3β; NF-Kb: nuclear factor-κB, STAT3: signal transducer and activator of transcription 3; TGF-h: Transforming growth factor-h; EGF: epidermal growth factor

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