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Original research

Emerging molecular markers for the prognosis of differentiated thyroid cancer patients

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ABSTRACT

Epithelial thyroid cancers are represented by the differentiated papillary and follicular thyroid carcinomas which, following dedifferentiation, are thought to give rise to the highly aggressive and incurable anaplastic thyroid carcinomas. Although derived from the same cell type, the different thyroid tumors show specific histological features, biological behavior and degree of differentiation as a consequence of different genetic alterations. Over the last few years, our knowledge regarding the molecular alterations underlying thyroid cell malignant transformation and cancer progression has considerably increased; however, the prognosis of differentiated thyroid cancer patients still relies on high-risk clinic-pathological variables. In particular, the actual staging systems provides only a rough prediction for cancer mortality and risk of recurrences, including in each risk group patients with highly different tumor-specific progression, disease-free interval and survival time. In order to improve DTC patient's risk stratification, both the European and the American Thyroid Associations proposed practical guidelines to integrate the actual staging systems with additional clinical features such as the tumor histological variant, the results of post-ablative whole body scan and the serum thyroglobulin levels. Despite that, patients within the same risk group still show a very heterogeneous behavior in terms of disease-free interval. As a consequence, the identification of new prognostic molecular biomarkers able to testify tumor aggressiveness is highly required. Here we'll review recently characterized new molecular markers potentially able to ameliorate the prognosis in DTC patients.

1. Thyroid cancer

Differentiated epithelial thyroid cancers (DTC) account for about 90–95% of all thyroid cancers and represent in the United States the fifth most common cancer in women [1,2]. The incidence of DTC has been increasing over the last decades, mainly as a result of the increasing ability to diagnose malignant transformation in small non-palpable nodules [3,4]. The DTC comprise two main histological entities, the follicular (FTC) and the papillary (PTC) thyroid carcinomas, which following dedifferentiation are assumed to generate the poorly DTC (PDTC) and the highly aggressive anaplastic thyroid carcinomas (ATC) unresponsive to any therapeutic agent [5,6]. Initial DTC treatment includes total thyroidectomy followed, in most patients, by adjuvant therapy with ^{131}I [7–9]. Patient's follow-up embraces radioiodine scanning 6–12 months after surgery, periodic ultrasound of the thyroid bed and cervical lymph node compartments, measurement of basal and recombinant human TSH-stimulated thyroglobulin (Tg) serum level [7–9].

2. Molecular events involved in thyroid cancer progression

Progression of human cancers, including DTC, is characterized by malignant cell acquisition of novel functional capabilities, which include self-sufficiency in growth signals, insensitivity to anti-growth signals, evasion of apoptosis, limitless replicative potential, sustained angiogenesis, tissue invasion and metastasis [10]. In particular, relevant molecular alterations encountered in thyroid cancer progression responsible for the acquisition of the above

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mentioned capabilities comprise gene rearrangements of tyrosine kinase receptors, such as the RET/PTC and NTRK1 (neurotrophic receptor-tyrosine kinase 1), or activating point mutations of proteins mediating cellular responses to growth and differentiation signals, including RAS and BRAF, or the oncogenic fusion protein PAX8-PPARγ, that suppresses wild-type PPARγ function in a dominant-negative manner [11,12]. Genomic instability, a hallmark of solid tumors including the DTC, is thought to represent the mean by which premalignant cells may acquire these new capabilities [10,13]. As in many other cancer types, also in thyroid cancer the number and the frequency of chromosomal abnormalities observed increase from the DTC to the poorly differentiated thyroid carcinoma (PDTC) and ATC [14,15]. It is worth to note that alterations in the expression of several mitotic kinases responsible for correct chromosome segregation have been described in both DTC and ATC, and are thought to play a central role in the establishment of genomic instability [14–17]. These include the Polo-like kinase 1 and the three members of the Aurora kinase family [18–20].

The conversion of early-stage thyroid tumors to more aggressive and invasive malignancies occurs through an epithelial-to-mesenchymal transition (EMT), which implies the loss of cell–cell contacts, remodeling of cytoskeleton, and the acquisition of a migratory phenotype [21,22]. Abnormal expression of integrins, Notch, MET, TGFβ, NF-κB, PI3K, TWIST1, matrix metalloproteinases (MMP), components of the urokinase plasminogen activating system and p21-activated kinase (Pak), involved in the EMT, have been identified in PTC progression [21,22].

3. The prognosis of differentiated thyroid cancer patients

Differentiated thyroid cancer patients usually have a favorable prognosis, with 10-years-survival rate of approximately 90%. Nevertheless, about 20% of them face the morbidity of disease recurrence and DTC-related deaths [1,2,23–25]. Despite the increasing knowledge of the molecular processes responsible for thyroid cell malignant transformation and cancer progression, to date, the prognosis of thyroid cancer patients still relies on high-risk clinic-pathological variables such as patient's age, tumor size, histology, lymph nodal or distant metastasis [26,27]. In particular, the TNM (Tumor size, lymph Node and distant Metastasis) system developed by the American Joint Committee on Cancer and the International Union Against Cancer is the most widely used staging system for thyroid cancer patients [28]. This staging system is strongly influenced by the patient's age, with those above 45 yr receiving a worse prognosis despite other factors being equal [28]. The TNM, as well as other staging systems proposed by recognized international organizations, are capable to make a rough prediction of the high or low risk of cancer mortality leaving, however, in the same risk group patients showing different disease-specific progression and survival time. Similarly, they fail to predict the risk of cancer recurrences [29]. Both the European (ETA) and the American Thyroid Associations (ATA) proposed practical guidelines to estimate the risk of recurrences in which the TNM parameters are integrated by additional clinical features such as the tumor histological variant, the results of post-ablative whole body scan and the serum Tg levels [9,24]. Despite that, patients included in the same risk group still show a very heterogeneous behavior in terms of disease-free interval. In addition, the stratification risk proposed by the ATA and the ETA is not accurate in predicting the long-term outcome in DTC patients, having a very low positive predictive value [30]. As a consequence, the identification of new prognostic molecular biomarkers able to testify tumor aggressiveness is highly required [31,32].

In this context, $BRAF^{V600E}$ mutation, the most prevalent genetic alteration observed in 29–87% of PTC and considered as an early genetic event in thyroid cancer progression received considerable attention as new prognostic marker in PTC [31–33]. However, its association with older age, the detection of the mutation in lymph node-metastasized PTC but not in primary tumors, and the recent demonstration that $BRAF^{V600E}$ is a secondary subclonal rather than a primary event in thyroid tumorigenesis, argue against the proposed primary tumorigenic role of $BRAF^{V600E}$ in PTC [32,34,35]. Nevertheless, several reports described the association of this mutation with factors related to poor prognosis, such as the presence of extrathyroidal extension, lymph node metastasis, advanced tumor stage, reduced disease-free interval and patient survival [31,33]. Despite the initial enthusiasm, a debate is actually ongoing about the clinical relevance of these findings [31,32]. In particular, some studies failed to associate the $BRAF^{V600E}$ with poor prognosis in PTC patients [33,34,36]. In addition, the frequency of BRAF mutation in PTC (on average 50%) is high compared with that of the poor outcomes (about 20%). On the other hand, a recent case-study performed on 977 patients affected by papillary microcarcinoma of the thyroid suggested that the highly aggressive tumors may arise in a subset of patients with the $BRAF^{V600E}$ [37]. From the currently available information it is evident that, based only on the analysis of the $BRAF^{V600E}$ mutation, a large percentage of patients would face the risk of over- or under-treatment. Is thus possible that in a near future the association of $BRAF^{V600E}$ with other clinical and/or molecular marker(s) could lead to a better prognostic definition of PTC patients.

The urokinase plasminogen activating system (uPAS) comprises the urokinase plasminogen activator (uPA), the cell membrane uPA receptor (uPAR) and the plasminogen activator inhibitor-1 (PAI-1) and -2 (PAI-2) [38]. It is involved on different aspects of human cancer progression including extracellular matrix degradation, activation of latent growth factors, malignant cells proliferation and spread to distant metastatic sites, and tumor neo-angiogenesis [38,39]. The prognostic value of uPAS has been demonstrated in different cancer types where overexpression of one or more uPAS components was shown to associate with a higher risk of relapse and poor clinical outcome [38,39]. This is particularly evident in breast cancer, in which uPA and PAI-1 show a predictive value stronger than patient age, tumor size, estrogen and progesterone receptors, HER-2/neu or p53 expression [40–44]. As a consequence, the American Society of Clinical Oncology has included both proteins among the recommended breast tumor markers for clinical use [45]. Over the last two decades, several studies investigated the expression of the uPAS in human thyrocytes [46–49]. Following malignant thyrocyte transformation the aberrant expression of uPAS components has been demonstrated [50–60]. More importantly, it has been evidenced that uPA, its cognate receptor uPAR and PAI-1 significantly associate with high-risk clinicopathological factors such as lymph node metastasis, higher TNM stage, shorter disease-free interval and overall survival [61–65]. Moreover, this association was found statistically stronger in stage I patients [34,61]. These findings may thus help to better define the prognosis, make informed therapeutic decisions and develop tailored prevention programs especially for stage I PTC patients, actually considered at low risk to develop disease recurrences. All together these observations indicate the potential prognostic value of uPAS components in thyroid cancer patients, and warrant further investigations on larger case-studies.

The estrogen receptors (ER) are members of the nuclear steroid receptor superfamily, and mediate cellular responses to estrogens which modulate growth, differentiation and function of different target tissues [66,67]. The ER function as ligand-dependent transcription factors, but they can also have a ligand-independent activity following phosphorylation by kinases [66,67]. Two different genes encode the ER, ERα and ERβ, showing different tissue