Thyroid cancer: an overview

Neoplasms derived from the epithelial follicular thyroid cell represent the most common endocrine malignancy being the fifth most common cancer in women in the USA (1, 2). Its annual incidence, roughly 1% of all new malignant diseases, has increased over the last decade, mainly because of our improved ability to diagnose malignant transformation in small thyroid nodules (3). The large majority of epithelial thyroid cancers is represented by the differentiated papillary (PTC) and follicular (FTC) thyroid carcinomas which, following differentiation, are thought to give rise to the highly aggressive and fatal anaplastic thyroid carcinomas (ATC) (4, 5). Although derived from the same cell type, the different tumours show specific histological features, biological behaviour and degree of differentiation as a consequence of different genetic alterations (6). In particular, early genetic mutations in thyroid cancer comprise gene rearrangements of tyrosine kinase receptors, such as 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 (6).

Importantly, the conversion of early-stage thyroid tumours to more aggressive and invasive malignancies occurs through an epithelial-to-mesenchymal transition (EMT), which implies the loss of cell–cell contacts, remodelling of cytoskeleton, and the acquisition of a migratory phenotype (7, 8). In fact, genetic alterations of integrins, Not...
ch, MET, TGFB, NF-xB, PI3K and p21-activated kinase (Pak), implicated in the EMT, have been identified in PTC progression (9, 10).

Thyroid nodules are very common, affecting 19% to 67% of the adult population (11, 12), but only about 5% of them harbour a malignant lesion. Therefore, the first aim in the clinical evaluation of a thyroid nodule is to exclude malignancy (1, 9). To date, fine-needle aspiration cytology (FNAC) represents the main diagnostic tool for the evaluation of both palpable and non-palpable thyroid nodules. It has to be mentioned, however, that FNAC suffers from a major diagnostic limit represented by follicular lesions (Thy3), in which the encountered cellular atypia are of indeterminate significance (13). Alternative diagnostic approaches have been put forward in order to overcome the diagnostic boundaries of FNAC (14-23), like patient's clinical characteristics, ultrasonography (US) parameters and identification of genetic alterations in fine-needle aspiration material; however, none of them turned out to improve presurgical Thy3 selection (14-23). In this context, some research groups have tried to develop gene-expression classifiers able to distinguish benign from malignant thyroid nodules. A very recent study reported the validation of a gene-expression classifier, comprising a panel of 167 genes evaluated by means of microarray, which showed a 92% sensitivity and negative predictive values ranging from 85% to 95% (24).

Total thyroidectomy followed by adjuvant therapy with 131I is the treatment of choice for most patients affected by differentiated thyroid carcinoma (DTC) (1, 13). After that, patients' follow-up includes radiiodine 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 thyroid-stimulating hormone (TSH) level (1, 13).

Although the prognosis of patients with DTC is favorable, with 10-years-survival rate of nearly 90%, about 20% of patients face the morbidity of disease recurrence and DTC-related deaths (1, 13, 25, 26). Despite our 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 tumour size, histology, lymph nodal or distant metastasis (27). As a consequence, the identification of molecular biomarkers strictly related to the risk of PTC relapse represents an attractive gain. Over the last few years BRAFV600E, the most prevalent genetic alteration in PTC, has been shown to associate with advanced tumour stage and a worse prognosis (28). However, conflicting findings have been reported by different studies, so its clinical utility as molecular prognostic marker remains doubtful (28, 29). In particular, the frequency of BRAF mutations in PTC is high (about 50%), compared with the poor outcomes (about 20%), and as a consequence a large percentage of patients would face the risk of over- or under-treatment based only on the analysis of BRAF mutation (28, 29). As below described, recent evidence have been provided that components of the uPAS can represent new valuable molecular prognostic markers for thyroid cancer patients (30-33).

The urokinase plasminogen activating system

The urokinase plasminogen activating system (uPAS) comprises the urokinase plasminogen activator (uPA), which converts in the pericellular environment the proenzyme plasminogen into the serine protease plasmin (34). The latter regulates a number of physiological processes requiring basement membrane (BM) and/or extracellular matrix (ECM) remodeling, such as wound healing, mammary gland development and its post-lactational involution, tissue regeneration and angiogenesis, as well as cancer progression and dissemination to distant sites (34). Physiological inhibition of the plasminogen activating system may occur either at the level of plasmin by α2-antiplasmin, or at the level of uPA, by the plasminogen activator inhibitor-1 and -2 (PAI-1 and PAI-2), which belong to the serine protease inhibitor superfamily of serpins (Figure 1) (34). The uPA is secreted from the cells as a single chain proenzyme (pro-uPA or sc-uPA) able to bind to a specific cell membrane glycolipid-anchored receptor, the uPA receptor (uPAR). The membrane-bound pro-uPA may be converted into the active uPA, a two chain molecule held together by a single disulfide bridge, by the action of different enzymes, including plasmin, cathepsin B and kallikrein. In view of the fact that also plasminogen binds to still undefined plasma membrane receptors, the occurrence in the same cells of both uPAR and plasminogen receptors may result in the formation of cell membrane-associated plasmin, leading to a localized pericellular ECM degradation. As mentioned, uPA activity is inhibited by PAI-1 and PAI-2, which interact with active uPA either free or bound to the uPAR. In the latter case, the complex is rapidly internalized by the cells and both uPA and its inhibitor are degraded, while the uPAR is recycled to the cell membrane (34).

Role of the uPAS in cancer progression

In cancer each uPAS member acts as a multi-tasking factor involved in all steps of tumor progression, from the local growth and spreading of malignant cells to migration and invasion of distant sites, as well as in tumor neoangiogenesis (Fig. 1) (34-37).
During cancer progression and metastatization malignant cells must be able to degrade and move through ECM and BM at the primary tumor site, to intra-vasate, extravasate and colonize target metastatic tissues. These capabilities are acquired through the increased expression and/or activity of proteolytic enzymes, especially plasmin and members of the MMPs family. In particular, plasmin is a broad spectrum enzyme which degrades several ECM and BM components, including fibronectin, laminin, vitronectin, type IV collagen, proteoglycans and fibrin, directly and/or through the activation of latent MMPs, such as proMMP-1, proMMP-2, proMMP-3, proMMP-9, proMMP-10 and proMMP-13 (34-40). Moreover, plasmin proteolytically activates motogenic growth factors, i.e. transforming growth factor-β, basic fibroblast growth factor and hepatocyte growth factor, that stimulate tumor cell migration. Importantly, due to its ability to move upon the plasma membrane and to bind integrins and vitronectin, the uPAR can accumulate in focal areas where plasmin generation is required, such as the leading and trailing edges of migrating cells. ECM proteolysis at the leading edge allows plasma membrane extension, while degradation of cell-ECM adhesions enables the release of the trailing edge (34-40).

Both uPA and plasmin promote tumor and endothelial cell proliferation by the activation or release of several mitogenic growth factors (i.e. epidermal growth factor, insulin growth factor, transforming growth factor-β, vascular endothelial growth factor and others). Moreover, uPAR, following uPA binding, can augment cell proliferation by triggering intracellular activation of protein kinase cascades (i.e. lymphocyte protein tyrosine kinase, lck; haematopoietic cell kinase, Hck; focal adhesion kinase, FAK, and mitogen-activated protein kinase, MAPK) (34).

As above mentioned, uPAS has been shown to play a major role in tumor neoangiogenesis (31-34). In endothelial cells uPAR is present at the leading migratory front of new microvessels, and the expression of uPA, uPAR and PAI-1 is enhanced by angiogenic stimuli like bFGF, VEGF or hypoxia. The locally produced plasmin in turn activates latent motogenic and proangiogenic factors bound to ECM, which promote endothelial cells mitosis, invasion, and capillary tube formation. Among these, the VEGF also induces vascular hyperpermeability, providing a route for metastasizing tumor cells and allowing diffusion of fibrinogen and other plasma proteins into the extracellular space. In the latter, the fibrinogen is converted to fibrin by plasmin leading to the formation of a transitional matrix, which provides an essential support for the migration of endothelial cells (34-41).

Clinical significance of uPAS components overexpression in cancer

Consistently with their role in cancer progression and metastasis, an augmented expression of uPA, its cognate receptor uPAR and PAI-1 has been observed in different malignant tumors (34-38, 42, 43). Furthermore, several studies demonstrated that high tumor tissue levels of uPA, uPAR and PAI-1 correlate with a poor prognosis in leukaemia, breast, lung, brain, esophageal, gastric, pancreatic, colorectal, hepatocellular, endometrial, ovarian, kidney, thyroid and bladder cancers (34-37, 42, 43). This is particularly evident in breast cancer, where uPA and PAI-1 have been identified as prognostic factors with a predictive value stronger than those of patient age, tumor size, estrogen and progesterone receptors, HER-2/neu or p53 expression (34, 35, 44-47). These clinical evidence prompted the American Society of Clinical Oncology to include uPA and PAI-1 among the recommended breast tumor markers for clinical use (48).

The above reported evidence recognize the uPAS as a major molecular player in human cancer progression and dissemination, and identify its components as suitable targets for anti-cancer therapy. In addition, the observation that uPA- or uPAR-deficient mice have normal embryonic development, growth, viability, and fertility indicates that pharmacological inhibition of uPAS...
is potentially devoid of major systemic side effects (34). In the past years, a number of different approaches have been attempted to neutralize the uPAS action in human cancers, mainly through the inhibition of uPA or uPAR expression or proteolytic activity and/or interaction. In general, preclinical studies proved the ability of different classes of functional uPA/uPAR inhibitors to cause regression of the invasive phenotype of different human malignant cell lines in vitro, and to reduce primary tumor burden and/or metastatic spreading in xenograft tumor models. Some of these inhibitors have recently entered clinical trials showing, in line with the expectation, their ability to induce disease stabilization (34). However, more exhaustive clinical investigations are still needed to prove the efficacy of uPAS inhibitors either in monotherapy or in combination with conventional or novel anti-cancer therapies.

The uPAS in thyroid cancer: clinical implications

Different studies have documented an increased expression of members of the MMP family and components of the uPAS during thyroid cancer progression (31-34, 49-57). An earlier study demonstrated the increased activity of uPA and MMPs in a follicular thyroid carcinoma cell line derived from lung metastasis (FTC-238), with respect to the less invasive clone derived from lymph node metastasis (FTC-133) (49). Another study reported the association of high uPAR expression with poorly differentiated and more aggressive PTC, indicating this protein as a putative prognostic factor (54). The levels of plasminogen activators and PAI-1 were also estimated by ELISA in the cytosolic fraction of malignant and benign thyroid tumor tissues and various non-cancer diseases of the gland (55). This work demonstrated that samples from patients with thyroid cancer displayed high levels of uPA and PAI-1, while samples from patients with benign thyroid diseases showed relatively low levels of uPA and PAI-1. Our group studied the expression profile of uPAS components in human PTC specimens compared to that of normal matched tissues by means of quantitative RT-PCR and Western blot (33). The results demonstrated that malignant transformation of the human thyroid cell is associated with the augmented expression, at both mRNA and protein levels, of uPA, uPAR, and PAI-1 (33). A different report analyzed the level of uPA and PAI-1 proteins in paired cytotoxic fractions of thyroid neoplasms and normal tissues by ELISA (58). Both proteins concentrations displayed the lowest values in adenomas and the highest in anaplastic carcinomas. Furthermore, uPA and PAI-1 were found higher in anaplastic versus well-differentiated thyroid cancers, as well as in tumor with extrathyroidal invasion or distant metastasis. More interestingly, the survival analysis revealed a significant impact of both uPA and PAI-1 on the progression-free survival rate (58). This was in agreement with a different study showing the association uPAR and disease survival of a case study comprising PTC, MTC, FTC and ATC patients (32). Finally, in recent studies, we have demonstrated that the increased gene expression of uPA and uPAR in PTC tissue is associated with tumor invasiveness, advanced stages and shorter disease-free interval (30, 31). This association was even more stronger when only stage I patients, actually considered at low risk of recurrences, were considered (30, 31). All together, these findings demonstrated in thyroid cancer a clear correlation between increased expression of uPAS components and some of the major prognostic factors for thyroid cancer, such as lymph nodal or distant metastases, tumor stage, disease-free interval and survival. This should warrant further large case studies to definitively validate the prognostic value of uPAS components in thyroid cancer patients in order to make more informed therapeutic decisions and to develop tailored prevention programs.

It is finally worth to mention that different experimental evidence indicate the uPAS as a suitable therapeutic target in thyroid cancer patients (59-60). In particular, it has been shown that the downregulation of uPA and uPAR expression or activity inhibits proliferation, migration and invasive capacity of PTC cells (59-60). If confirmed, these findings could open a new scenario for the treatment, either in monotherapy or in combination with novel anti-cancer agents, of those thyroid cancer patients affected by poorly differentiated or undifferentiated thyroid carcinoma unmanageable with radioiodine treatment or any available antimitoric drugs (61-63).

References


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