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review

The urokinase plasminogen activating system in thyroid cancer: clinical implications

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SUMMARY: **The urokinase plasminogen activating system in thyroid cancer: clinical implications.**

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The urokinase plasminogen activator (uPA) system (uPAS) comprises the uPA, its cell membrane receptor (uPAR) and two specific inhibitors, the plasminogen activator inhibitor 1 (PAI-1) and 2 (PAI-2). The uPA converts the plasminogen in the serine protease plasmin, involved in a number of physiopathological processes requiring basement membrane (BM) or extracellular matrix (ECM) remodelling, including tumor progression and metastasis. The tumor-promoting role of PAS is not limited to the degradation of ECM and BM required for local diffusion and spread to distant sites of malignant cells, but widens to tumor cell proliferation, adhesion and migration, intravasation, growth at the metastatic site and neoangiogenesis. The relevance of uPAS in cancer progression has been confirmed by several studies which documented an increased expression of uPA, uPAR and PAI-1 in different human malignancies, and a positive correlation between the levels of one or more of them and a poor prognosis. For these reasons, the uPAS components have aroused considerable interest as suitable targets for anticancer therapy, and several pharmacological approaches aimed at inhibiting the uPA and/or uPAR expression or function in preclinical and clinical settings have been described. In the present manuscript, we will first glance at uPAS biological functions in human cancer progression and its clinical significance in terms of prognosis and therapy. We will then review the main findings regarding expression and function of uPAS components in thyroid cancer tissues along with the experimental and clinical evidence suggesting its potential value as molecular prognostic marker and therapeutic target in thyroid cancer patients.

KEY WORDS: Plasminogen activating system - Urokinase - Thyroid - Cancer - Prognosis - Therapy.

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 dedifferentiation, 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 PPAR 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-

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ch, MET, TGFβ, NF-κB, 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 atypias 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 ^{131}I is the treatment of choice for most patients affected by differentiated thyroid carcinoma (DTC) (1, 13). After that, patients' follow-up includes 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 serum 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 $BRAF^{V600E}$, 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) remodelling, 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).

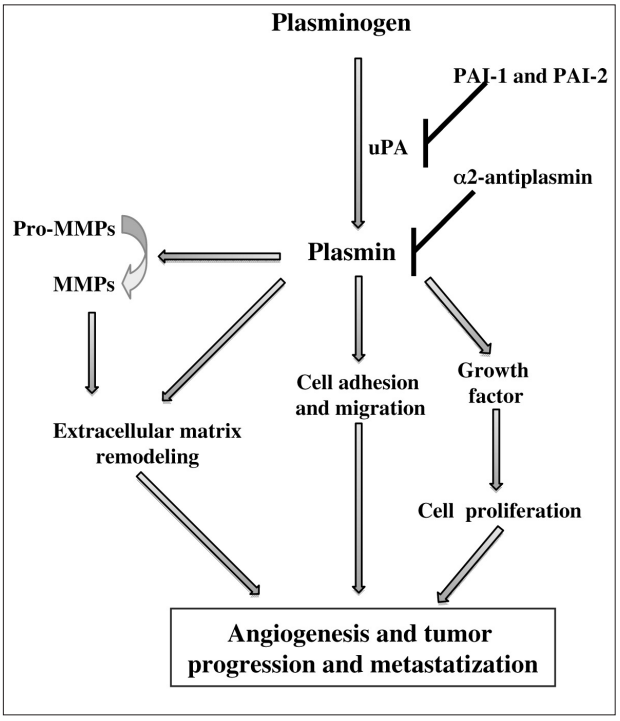

Fig. 1 - Schematic representation of the main functions of the urokinase plasminogen activating system in cancer progression and metastatization and angiogenesis.

During cancer progression and metastatization malignant cells must be able to degrade and move through ECM and BM at the primary tumor site, to intravasate, 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 of 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 tumour 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 thyrocyte 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 cytosolic 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 antimitotic drugs (61-63).

References

1. Sherman SI. Thyroid carcinoma. Lancet 2003;361:501-11.
2. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer Statistics, 2009. Ca Cancer J Clin 2009;59:225-49.
3. Trimboli P, Ulisse S, Graziano FM, Marzullo A, Ruggieri M, Calvanese A, Piccirilli F, Cavaliere R, Fumarola A, D'Armiento M. Trend in thyroid carcinoma size, age at diagnosis, and histology in a retrospective study of 500 cases diagnosed over 20 years. Thyroid 2006;16:1151-5.
4. Kinder B K. Well differentiated thyroid cancer. Curr Opin Oncol 2003;15:71-7.
5. Pasieka JL. Anaplastic thyroid cancer. Curr Opin Oncol 2003;15:78-83.

6. Nikiforov, YE (2009) Diagnostic pathology and molecular genetics of the thyroid. Lippincott Williams & Wilkins, Philadelphia.
7. Motti ML, Califano D, Troncone G, De Marco C, Migliaccio I, Palmieri E, Pezzullo L, Palombini L, Fusco A, Viglietto G. Complex regulation of the cyclin-dependent kinase inhibitor p27kip1 in thyroid cancer cells by the PI3K/AKT pathway: regulation of p27kip1 expression and localization. Proc Natl Acad Sci U S A 2007;104:2803-8.
8. Huber MA, Kraut N, Beug H. Molecular requirements for epithelial-mesenchymal transition during tumor progression. Curr Opin Cell Biol 2005;17:548-58.
9. Vasko V, Espinosa AV, Scouten W, He H, Auer H, Liyanarachchi S, Larin A, Savchenko V, Francis GL, de la Chapelle A, Saji M, Ringel MD. Gene expression and functional evidence of epithelial-to-mesenchymal transition in papillary thyroid carcinoma invasion. Proc Natl Acad Sci USA 2007;104:2803-8.
10. Pasquali D, Santoro A, Bufo P, Conzo G, Deery WJ, Renzullo A, Accardo G, Sacco, Bellastella A, Pannone G. Upregulation of Endocrine Gland-Derived Vascular Endothelial Growth factor in Papillary thyroid Cancers Displaying Infiltrative Patterns, Lymph Node Metastases, and Braf mutation. Thyroid 2011;21:391-9.
11. Roman SA. Endocrine tumors: evaluation of the thyroid nodule. Curr Opin Oncol 2003;15:66-70.
12. Welker MJ, Orlov D. Thyroid nodules. Am Fam Phys 2003;67:559-66.
13. American Thyroid Association (ATA) Guidelines Taskforce on Thyroid Nodules and Differentiated Thyroid Cancer, Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL, Mandel SJ, Mazzaferri EL, McIver B, Pacini F, Schlumberger M, Sherman SI, Steward DL, Tuttle RM. Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. Thyroid 2009;19:1167-214.
14. Rago T, Di Coscio G, Basolo F, Scutari M, Elisei R, Berti P, Miccoli P, Romani R, Faviana P, Pinchera A, Vitti P. Combined clinical, thyroid ultrasound and cytological features help to predict thyroid malignancy in follicular and Hurtle cell thyroid lesions: results from a series of 505 consecutive patients. Clin Endocrinol 2007;66:13-20.
15. Sorrenti S, Trimboli P, Catania A, Ulisse S, De Antoni E, D'Armiento M. Comparison of malignancy rate in thyroid nodules with cytology of indeterminate follicular or indeterminate Hürthle cell neoplasm. Thyroid 2009;19:355-60.
16. Trimboli P, Ulisse S, D'Alò M, Solari F, Fumarola A, Ruggieri M, De Antoni E, Catania A, Sorrenti S, Nardi F, D'Armiento M. Analysis of clinical, ultrasound and colour flow-Doppler characteristics in predicting malignancy in follicular thyroid neoplasms. Clin Endocrinol 2008;69:342-44.
17. Trimboli P, Sorrenti S. Low value of color flow-doppler in predicting malignancy of thyroid follicular neoplasms. Diagn Cytopathol 2009;37:391-2.
18. Trimboli P, Condorelli E, Catania A, Sorrenti S. Clinical and ultrasound parameters in the approach to thyroid nodules cytologically classified as indeterminate neoplasm. Diagn Cytopathol 2009;37:785-5.
19. Troncone G, Volante M, Iaccarino A, Zeppa P, Cozzolino I, Malapelle U, Palmieri EA, Conzo G, Papotti M, Palombini L. Cyclin D1 and D3 overexpression predicts malignant behaviour in thyroid fine-needle aspirates suspicious for Hurthle cell neoplasms. Cancer 2009;117:522-9.
20. Sapio MR, Posca D, Raggioli A, Guerra A, Marotta V, Deandrea M, Motta M, Limone PP, Troncone G, Caleo A, Rossi G, Fenzi G, Vitale M. Detection of RET/PTC, TRK and BRAF mutations in preoperative diagnosis of thyroid nodules with indeterminate cytological findings. Clin Endocrinol 2007;66:678-83.
21. Salvatore G, Giannini R, Faviana P, Caleo A, Migliaccio I, Fagin JA, Nikiforov YE, Troncone G, Palombini L, Basolo F, Santoro M. Analysis of BRAF point mutation and RET/PTC rearrangements refines the fine-needle aspiration diagnosis of papillary thyroid carcinoma. J Clin Endocrinol Metab 2004;89:5175-80.
22. Leidig-Bruckner G, Cichorowski G, Sattler P, Bruckner T, Sattler B. Evaluation of thyroid nodules – combined use of 99mTc-methylisobutylnitrile scintigraphy and aspiration cytology to assess risk of malignancy and stratify patients for surgical or nonsurgical therapy – a retrospective study. Clin Endocrinol 2012;76:749-58.
23. Deandreis D, Al Ghuzian A, Auperin A, Vielh P, Caillou B, Chami L, Lumbroso J, Travagli JP, Hartl D, Baudin E, Schlumberger M, Leboulleux S. Is 18F-fluorodeoxyglucose-PET/CT useful for the presurgical characterization of thyroid nodules with indeterminate fine needle aspiration cytology? 2012;22:165-72.
24. Alexander EK, Kennedy GC, Baloch ZW, Cibas ES, Chudova D, Diggans J, Friedman L, Kloos RT, LiVolsi VA, Mandel SJ, Raab SS, Rosai J, Steward DL, Walsh PS, Wilde JI, Zeiger MA, Lanman RB, Haugen BR. Preoperative diagnosis of benign thyroid nodules with indeterminate cytology. N Engl J Med 2012;367:705-15.
25. Passler C, Scheuba C, Prager G, Kaczirek K, Kaserer K, Zettinig G, Niederle B. Prognostic factors of papillary and follicular thyroid cancers: differences in an iodine-replete endemic goiter region. Endocr-Relat Cancer 2004;11:131-9.
26. Eustatia-Rutten CF, Corssmit EP, Biermasz NR, Pereira AM, Romijn JA, Smit JW. Survival and death causes in differentiated thyroid carcinoma. J Clin Endocrinol Metab 2006;91:313-9.
27. Falvo L, Catania A, D'Andrea V, Marzullo A, Giustiniani MC, De Antoni E. Prognostic importance of histologic vascular invasion in papillary thyroid carcinoma. Ann Surg 2005;241:640-6.
28. Xing M. BRAF mutation in papillary thyroid cancer: pathogenic role, molecular bases, and clinical implications. Endocr Rev 2007;28:742-62.
29. Handkiewicz-Junak D, Czarniecka A, Jarząb B. Molecular prognostic markers in papillary thyroid cancer: current status and future directions. Mol Cell Endocrinol 2010;322:8-28.
30. Ulisse S, Baldini E, Sorrenti S, Barollo S, Gnessi L, Catania A, Pelizzo MR, Nardi F, Mian C, De Antoni E, D'Armiento M, Frati L. High expression of the urokinase plasminogen activator and its cognate receptor associates with advanced stages and reduced disease-free interval in papillary thyroid carcinoma. J Clin Endocrinol Metab 2011;96:504-8.
31. Ulisse S, Baldini E, Sorrenti S, Barollo S, Prinzi N, Catania A, Nesca A, Gnessi L, Pelizzo MR, Mian C, De Vito C, Calvanese A, Palermo S, Persechino S, De Antoni E, D'Armiento M. In papillary thyroid carcinoma BRAFV600E is associated with increased expression of the urokinase plasminogen activator and its cognate receptor, but not with disease-free interval. Clin Endocrinol 2012, In press, doi: 10.1111/j.1365-2265.2012.04465.x.
32. Buergy D, Weber T, Maurer GD, Mudduluru G, Medved F, Leupold JH, Brauckhoff M, Post S, Dralle H, Allgayer H. Urokinase receptor, MMP-1 and MMP-9 are markers to differentiate prognosis, adenoma and carcinoma in thyroid malignancies. Int J Cancer 2009;125:894-901.
33. Ulisse S, Baldini E, Toller M, Marchioni E, Giacomelli L, De Antoni E, Ferretti E, Marzullo A, Graziano FM, Trimboli P, Biordi L, Curcio F, Gulino A, Ambesi-Impiombato FS, D'Armiento M. Differential Expression of the Components of the Plasminogen Activating System in Human Thyroid Tumor Derived Cell Lines and Papillary Carcinomas. Eur J Cancer 2006;42:2631-8.

34. Ulisse S, Baldini E, Sorrenti S, D'Armiento M. The urokinase plasminogen activator system: a target for anti-cancer therapy. Curr Cancer Drug Tar 2009;9:32-71.
35. Duffy MJ. The urokinase plasminogen activator system: role in malignancy. Curr Pharm Des 2004;10:39-49.
36. Choong PFM, Nadesapillai APW. Urokinase plasminogen activator system: a multifunctional role in tumor progression and metastasis. Clin Orthop Relat Res 2003;415S: S46-S58.
37. Ulisse S, Baldini E, Mottolese M, Sentinelli S, Gargiulo P, Brancato V, Sorrenti S, Di Benedetto A, De Antoni E, D'Armiento M. Increased expression of urokinase plasminogen activator and its cognate receptor in human seminomas. BMC Cancer 2010;10:151.
38. Andreasen PA, Kjøller L, Christensen L, Duffy MJ. The Urokinase-Type Plasminogen Activator System in Cancer Metastasis: A Review. Int J Cancer 1997;72:1-22.
39. Blasi F, Carmeliet P. uPAR: a Versatile Signalling Orchestrator. Nat Rev Mol Cell Biol 2002;3:932-43.
40. Estricher A, Mühlhauser J, Carpentier JL, Orci L, Vassalli JD. The Receptor for Urokinase Type Plasminogen Activator Polarizes the Expression of the Protease to the Leading Edge of Migrating Monocytes and Promotes Degradation of Enzyme Inhibitor Complexes. J. Cell Biol 1990;111:783-92.
41. Binder BR, Mihaly J, Prager GW. uPAR–uPA–PAI-1 Interactions and Signaling: a Vascular Biologist's View. Thromb Haemost 2007;97:336-42.
42. de Bock CE, Wang Y. Clinical significance of urokinase-type plasminogen activator receptor (uPAR) expression in cancer. Med Res Rev 2004;24:13-39.
43. Duffy MJ. Urokinase-type plasminogen activator: a potent marker of metastatic potential in human cancer. Biochem Soc Trans 2002;30:207-210.
44. Schmitt M, Harbeck N, Thomssen C, Wilhelm O, Magdolen V, Reuning U, Ulm K, Höfler H, Jänicke F, Graeff H. Clinical impact of the plasminogen activation system in tumor invasion and metastasis: prognostic relevance and target for therapy. Thromb Haemost 1997;78:285-96.
45. Look MP, van Putten WL, Duffy MJ, Harbeck N, Christensen IJ, Thomssen C, Kates R, Spyratos F, Fernö M, Eppenberger-Castori S, Fred Sweep CG, Ulm K, Peyrat JP, Martin PM, Magdelenat H, Brünner N, Duggan C, Lisboa BW, Bendahl PO, Quillien V, Daver A, Ricolleau G, Meijer-van Gelder M, Manders P, Edward Fiets W, Blankenstein M, Broët P, Romain S, Daxenbichler G, Windbichler G, Cufer T, Borstnar S, Kueng W, Beex L, Klijn J, O'Higgins N, Eppenberger U, Jänicke F, Schmitt M, Foekens J. Pooled Analysis of Prognostic Impact of Urokinase-type Plasminogen Activator and its Inhibitor PAI-1 in 8377 Breast Cancer Patients. J Natl Cancer Inst 2002;94:116-28.
46. Jänicke F, Prechtl A, Thomssen C, Harbeck N, Meisner C, Untch M, Sweep CG, Selbmann HK, Graeff H, Schmitt M; German N0 Study Group. Randomized Adjuvant Chemotherapy Trial in High-risk, Lymph Node-negative Breast Cancer Patients Identified by Urokinase-type Plasminogen Activator and Plasminogen Activator Inhibitor Type 1. J Natl Cancer Inst 2001;93:913-20.
47. Foekens JA, Peters HA, Look MP, Portengen H, Schmitt M, Kramer MD, Brünner N, Jänicke F, Meijer-van Gelder ME, Henzen-Logmans SC, van Putten WL, Klijn JG. The Urokinase System of Plasminogen Activation and Prognosis in 2780 Breast Cancer Patients. Cancer Res 2000;60:636-43.
48. Harris L, Fritsche H, Mennel R, Norton L, Ravdin P, Taube S, Somerfield MR, Hayes DF, Bast RC Jr, American Society of Clinical Oncology. American Society of Clinical Oncology 2007 update of recommendations for the use of tumor markers in breast cancer. J Clin Oncol 2007;25:5287-312.
49. Packman KS, Demeure MJ, Doffek KM, Wilson SD. Increased Plasminogen Activator and Type IV Collagenase Activity in Invasive Follicular Thyroid Carcinoma Cells. Surgery 1995;118:1011-6.
50. Baldini E, Toller M, Graziano FM, Russo FP, Pepe M, Biordi L, Marchioni E, Curcio F, Ulisse S, Ambesi-Impiombato FS, D'Armiento M. Expression of matrix metalloproteinases and their specific inhibitors (TIMPs) in normal and different human thyroid tumor cell lines. Thyroid 2004;14:881-8.
51. Sid B, Langlois B, Sartelet H, Bellon G, Dedieu S, Martiny L. Thrombospondin-1 enhances human thyroid carcinoma cell invasion through urokinase activity. Int J Biochem Cell Biol 2008;40:1890-900.
52. Chu QD, Hurd TC, Harvey S, Martinick M, Markus G, Tan D, Gibbs JF, Loree T. Overexpression of urinary plasminogen activator (uPA) protein and mRNA in thyroid carcinogenesis. Diag Mol Pathol 2004;13:241-6.
53. Sid B, Dedieu S, Delorme N, Sartelet H, Rath GM, Bellon G, Martiny L. Human thyroid carcinoma cell invasion is controlled by the low density lipoprotein receptor-related protein-mediated clearance of urokinase plasminogen activator. Int J Biochem Cell Biol 2006;38:1729-40.
54. Ito Y, Takeda T, Kobayashi T, Wakasugi E, Tamaki Y, Umeshita K, Monden T, Shimano T, Monden M. Plasminogen Activation System in Active Even in Thyroid Tumors; an Immunohistochemical Study. Anticancer Res 1996;16:81-9.
55. Kim SJ, Shiba E, Taguchi T, Tsukamoto F, Miyoshi Y, Tanji Y, Takai S, Noguchi S. uPA Receptor Expression in Benign and Malignant Thyroid Tumors. Anticancer Res 2002;22:387-93.
56. Smit JW, van der Pluijm G, Romijn HA, Löwik CW, Morreau H, Goslings BM. Degradation of extracellular matrix by metastatic follicular thyroid carcinoma cell lines: role of the plasmin activation system. Thyroid 1999;9:913-9.
57. Zanetti A, Stoppacciaro A, Marzullo A, Ciabatta M, Fazioli F, Prat M, Comoglio PM, Baroni CD, Ruco LP. Expression of Met protein and urokinase-type plasminogen activator receptor (uPA-R) in papillary carcinoma of the thyroid. J Pathol 1998;186:287-91.
58. Horvatić Herceg G, Herceg D, Kralik M, Bence-Zigman Z, Tomić-Brzac H, Kulić A. Urokinase-Type Plasminogen Activator and its Inhibitor in Thyroid Neoplasms: a Cytosol Study. Wien Klin Wochenschr 2006;118:601-9.
59. Nowicki TS, Kummer NT, Iacob C, Suslina N, Schaefer S, Schantz S, Shin E, Moscatello AL, Tiwari RK, GeliebterJ. Inhibition of uPAR and uPA reduces invasion in papillary thyroid carcinoma cells. Laryngoscope 2010;120:1383-90.
60. Nowicki TS, Zhao H, Darzynkiewicz Z, Moscatello A, Shin E, Schantz S, Tiwary RK, Geliebter J. Downregulation of uPAR inhibits migration, invasion, proliferation, FAK/PI3K/Akt signaling and induces senescence in papillary thyroid carcinoma cells. Cell Cycle 2011;10:100-7.
61. Nowicki TS, Moscatello AL, Shin E, Schantz S, Tiwary RK, Geliebter J. The urokinase plasminogen activator system in metastatic papillary thyroid carcinoma: a potential therapeutic target. J Clin Endocrinol Metab 2011;96:3062-4.
62. Arlot-Bonnemains Y, Baldini E, Martin B, Delcros JG, Toller M, Curcio F. Ambesi-Impiombato FS, D'Armiento M, Ulisse S. Effects of the Aurora kinase inhibitor VX-680 on anaplastic thyroid cancer-derived cell lines. Endocr-Relat Cancer 2008;15:559-68.
63. Antonelli A, Fallahi P, Ulisse S, Ferrari SM, Minuto M, Saraceno G, Santini F, Mazzi V, D'Armiento M, Miccoli P. New targeted therapies for anaplastic thyroid cancer. Anti-Cancer Ag Med Chem 2012;12:87-93.