

Urokinase-Type Plasminogen Activator Receptor: A Beacon of Malignancy?

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Discriminating between benign and malignant disease is a pivotal diagnostic issue in the care of women with pelvic masses. Identification of serum biomarkers that can reliably make the distinction would aid in the proper referral for patient care and may provide leads in the development of early detection strategies.

In this issue of *Clinical Cancer Research*, Henic and colleagues (1) show that soluble forms of urokinase-type plasminogen activator receptor represent a promising new tool in the development of a discriminatory biomarker panel that can influence patient care.

Epithelial ovarian cancer is one of the three tumor types that were selected for studies during the pilot phase of The Cancer Genome Atlas project, a NIH initiative that will use large-scale genome analysis technologies to determine all of the important genomic changes involved in cancer. Ovarian carcinoma is a low-incidence disease with approximately 22,000 new cases diagnosed annually in the United States (2). However, the case-to-fatality ratio is exceedingly high for ovarian cancer, making it three more lethal than breast cancer and the most deadly gynecologic malignancy in developed countries. The lack of effective screening methods frequently results in the majority of patients being diagnosed at an advanced stage when the opportunity for a surgical cure is drastically reduced. Conversely, organ-confined disease is associated with an excellent prognosis and a 5-year survival rate >90%. Furthermore, whereas most patients initially respond to standard chemotherapeutic regimens, the majority ultimately relapses with chemoresistant disease (3). Therefore, to establish optimal management strategies for these patients, efforts are needed to develop biomarkers for early detection of disease, therapeutic prognosis, response to treatment, and disease recurrence. Given the survival advantage of early detection in ovarian cancer, a significant effort is under way to identify biomarkers for this purpose.

CA125 is an ovarian cancer serum biomarker clinically approved for following the response to treatment, predicting prognosis after treatment, and detecting the recurrence of disease (4). However, its potential role for the early detection of ovarian cancer is controversial because randomized screening trials of asymptomatic women with ovarian cancer mortality as the end point have yet to be completed. The largest of two ongoing trials with this end point and a CA125 component (5, 6) required 200,000 postmenopausal women and screening for 10 years due to the low incidence of ovarian cancer. Both trials will be completed in 2014. Whereas the effectiveness of CA125 screening and the exact method for interpreting CA125 remain an open issue, it is likely that a blood biomarker panel for ovarian screening would include CA125. There are intensive discovery efforts under way for biomarkers that complement CA125 or elevate earlier than CA125 in clinically undetected disease.

For a candidate to serve as an early-stage ovarian cancer biomarker, it must fulfill a number of criteria. Ideally, adding the candidate to a panel should significantly increase the sensitivity of the panel at the same specificity. The minimum requirement for a viable ovarian cancer screening test is a positive predictive value of 10% (i.e., at most 10 operations for each ovarian cancer detected). Whereas the bar for screening sensitivity is less clear, a reasonable value is 75%. With this value, a positive predictive value of 10%, and an annual incidence of 1 in 2,300, as occurs in postmenopausal women, the specificity required is 99.6%. Based on the U.K. trial results, a first-line blood test with 2% of subjects annually receiving a second-line ultrasound test produces an overall specificity of 99.8% and a positive predictive value exceeding 20% (7). Therefore, setting the blood test specificity at 98% empirically results in an acceptable positive predictive value. However, a prospective trial is required to establish the sensitivity for early-stage clinically undetected disease. Before such a long-term effort is made, an estimate of sensitivity for preclinical disease should be calculated using precious samples from cases diagnosed during previous long-term screening trials. Before consuming such samples, the sensitivity (at 98% specificity) of a new candidate biomarker in clinically diagnosed cases should be obtained, along with the increase in sensitivity above an existing panel (e.g., CA125 alone).

In this issue, Henic and colleagues (1) make important strides toward establishing uPAR as an ovarian cancer

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biomarker. They present evidence that cleaved forms of the urokinase-type plasminogen activator (uPA) receptor (uPAR) have diagnostic potential in distinguishing between benign and malignant adnexal masses. The uPA system is involved in various cellular processes that contribute to the development, angiogenesis, inflammation, and metastasis of tumors (8, 9). The system consists mainly of the serine protease uPA, its cell membrane-associated receptor uPAR, a substrate (plasminogen), and two plasminogen activator inhibitors (PAI-1; ref. 10; Fig. 1). On binding to uPAR, uPA catalyzes the cleavage of plasminogen to form plasmin, which can either directly degrade basement membrane and extracellular matrix or activate other zymogen proteases such as procollagenase (8). In this way, uPAR can localize enzyme activity at the cell surface within the tumor microenvironment, thereby mediating proteolysis that can facilitate migration of tumor cells.

uPAR is bound to the cell surface by a glycosyl phosphatidylinositol linkage, but various forms of soluble uPAR (suPAR) can also be generated by cleavage of the linker regions between the cysteine domains of uPAR. uPA itself can cleave uPAR between DI and DII, liberating the ligand-binding domain DI (suPAR-I) and thereby inactivating the binding potential of uPAR for uPA. In addition, uPAR can be shed from cells by cleavage at the lipid anchor by phospholipases or proteases, liberating full-length uPAR (I-III) or uPAR (II-III; see Fig. 1; ref. 8). Henic et al. (1) measured the levels of all three soluble forms of uPAR (suPAR I-III, II-III, and I) in a cohort of 355 women with benign and malignant adnexal masses. They found that all suPAR forms as well as CA125 were statistically significant in univariate analysis discriminating between benign, borderline, and invasive tumors. Importantly, the combination of CA125 and suPAR(I-III) + suPAR(II-III)

discriminated between malignant and benign tumors with an area under the curve of 0.94. The specificity at 85% sensitivity for invasive versus benign disease seems to be sufficiently high to be clinically useful for differential diagnosis of adnexal masses. Some caution is warranted, however, because the parameters in the proportional odds model for combining suPAR and CA125 were based on the same data from which the sensitivity and specificity were estimated, introducing the possibility of overfitting bias (11).

Another important observation made by Henic and colleagues is that whereas none of the suPARs seemed to correlate with histologic grade or stage, they found no differences in plasma levels of suPAR(I-III) + suPAR(II-III), suPAR(I-III), or uPAR(I) between patients with endometriosis and other benign ovarian cysts. All were low compared with ovarian cancers, a distinction that has plagued CA125 particularly in premenopausal women. Moreover, high preoperative levels of uPAR(I) are an independent predictor of poor prognosis.

There are at least two clinical implications of these findings. First, there is currently no accepted method to predict whether an adnexal mass is likely to be malignant before surgery, although recent results on combining HE4 and CA125 show promise (12). The study by Henic shows that suPAR(I-III) + suPAR(II-III) and CA125 clearly discriminated between invasive (all stages or only early stage) and benign tumors. These promising results pave the way for the development of a composite biomarker, perhaps in conjunction with improved imaging modalities that may have the necessary sensitivity and specificity to select those patients with ovarian masses that should be referred to centers with gynecologic oncologist. Aggressive surgical efforts have a positive effect on survival of patients with advanced disease (13), and this effort is most

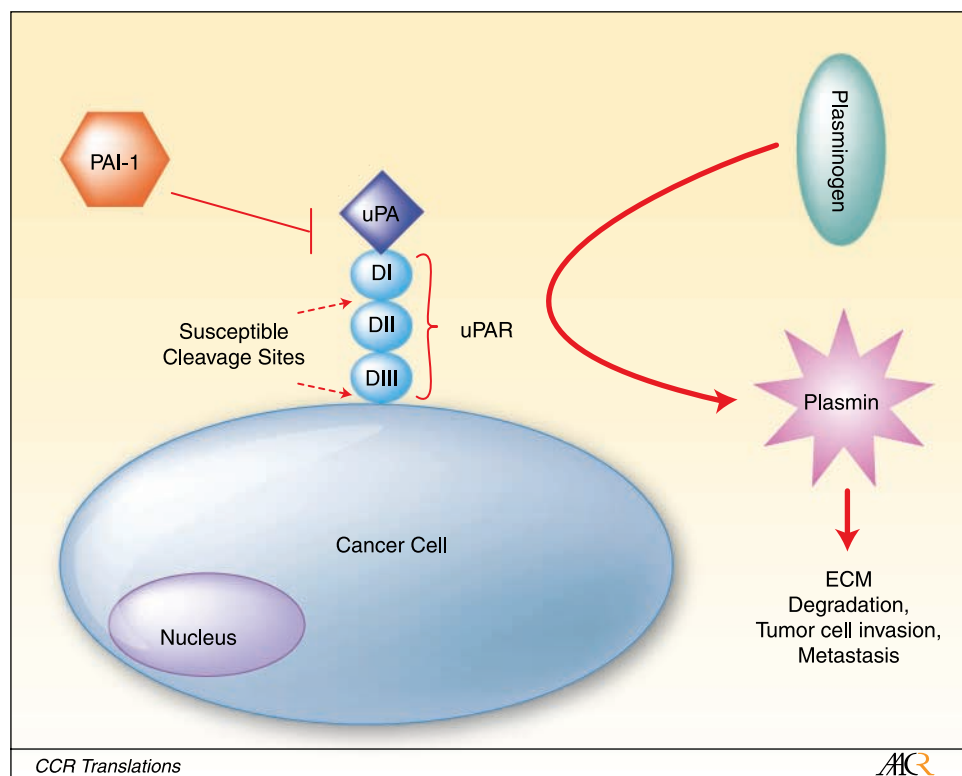


Fig. 1. The uPAR is a glycosylated extracellular receptor that is covalently linked to the outer layer of the cell membrane via a glycosyl phosphatidylinositol anchor. uPAR consists of three cysteine-rich domains (DI, DII, and DIII) connected by two short linker regions. The NH₂ terminus of the uPAR domain I is the primary site for the binding of uPA.

often achieved successfully by gynecologic oncologists (14). Therefore, the development of a diagnostic tool to help triage patients for appropriate care could greatly affect morbidity and outcomes. Second, the results from this study seem to warrant further research on this candidate in samples from populations relevant to screening. Henic found that the combination of suPAR(I-III) + suPAR(II-III) and CA125 differentiates early-stage tumors from benign adnexal masses better than either marker alone. Whereas this population is not ideal for making claims about a candidate's screening potential, it is a good first-pass surrogate. The results strongly argue for further investigation as an early detection biomarker,

with the next steps examining comparisons to healthy controls, and only then investigating longitudinal samples from screening trials.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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