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Original contribution

Persistent uroplakin expression in advanced urothelial carcinomas: implications in urothelial tumor progression and clinical outcome

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Summary As the terminal differentiation products of human urothelium, uroplakins (UPs) would be expected to diminish during urothelial tumorigenesis. Surprisingly, recent studies found UPs to be retained even by well-advanced urothelial carcinomas, suggesting that the loss of UPs does not strictly parallel urothelial transformation. Little is known, however, about whether the status of UPs is associated with a particular pathologic parameter, the tumor's biological behavior, or patient outcome. Here we assessed UP expression by immunohistochemistry on tissue arrays from 285 patients with bladder urothelial carcinomas or nontumor conditions. UPs were expressed in all 9 normal urothelial specimens, 63 of 74 (85%) patients with non–muscle-invasive urothelial carcinomas on transurethral resection, 104 of 202 (51.5%) patients who underwent radical cystectomy for advanced urothelial carcinomas, and 33 of 50 (66%) lymph node metastases. Normally associated with urothelial apical surface, UPs were localized aberrantly in tumors, including microluminal, basal-laminal, cytoplasmic, or uniform patterns. In non–muscle-invasive diseases, there was no association between UP expression and disease recurrence, progression, or mortality. In contrast, in invasive diseases, absent UP expression was significantly associated with advanced pathologic stage, lymph node metastases, disease recurrence, and bladder cancer–specific mortality ($P = .042$, $P = .035$, $P = .023$, and $P = .022$, respectively) in univariate analyses. Furthermore, UP status was independent of key cell-cycle regulators, including p53, pRb, p27, and cyclin D1, thus excluding a functional link between these 2 groups of proteins. Our data demonstrate

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for the first time that persistent UP expression is associated with a favorable clinical outcome and that UPs may be used as adjunct markers for predicting the prognoses of patients with invasive and metastatic bladder carcinomas. Our results also suggest that UP-positive and -negative carcinomas have different clonal origins or may be derived from different cancer stem cells.

1. Introduction

Urothelial carcinomas of the bladder are among the most common human malignancies, ranked fourth in incidence in all male cancers and ninth in all female cancers in the United States. According to the American Cancer Society, more than 61 000 new cases were diagnosed in 2006, of which 13 000 will die of this disease [1]. Globally, approximately 336 000 new cases are diagnosed annually, resulting in 132 000 deaths [2]. Fortunately, not all urothelial carcinomas carry the same risk of reaching the incurable stage. In fact, there is increasing evidence to suggest that at least 2 major carcinoma variants exist: the low-grade, superficial papillary tumors and the high-grade invasive tumors [3-9]. The former frequently recur, but relatively few of them progress to muscle-invasive stages. In contrast, the latter are derived mainly from flat carcinoma in situ (CIS) and, less often, from high-grade papillary tumors. They follow an aggressive course, with at least half of the cases eventually progressing with local and distant metastases [10]. This occurs in an unpredictable manner and despite the complete removal of the primary carcinomas by radical cystectomy, thus presenting a major challenge in curing this disease [11]. In this regard, it is critically important to be able to predict the biological behavior and the likelihood of progression of a given carcinoma at presentation, as this will greatly facilitate an appropriate treatment decision. However, the number and type of prognostic markers available for this purpose remain insufficient.

Uroplakins (UPs) are a group of integral membrane proteins that are synthesized during an advanced stage of human urothelial differentiation [12]. Thus far, 4 major UPs (UPIa, Ib, II, and IIIa) and one minor UP (UPIIIb) have been identified [12,13]. Together, these proteins constitute the major protein building blocks of the urothelial plaques, also named *asymmetric unit membranes* (or AUMs), that cover more than 90% of the urothelial surface [14,15]. Topologically, UPIa and Ib span the plasma membrane 4 times and belong to a growing family of structurally conserved but functionally elusive proteins, all possessing 4 transmembrane domains (tetraspanins). UPII and IIIa, however, contain only one transmembrane domain, which divides a large luminal domain and a smaller cytoplasmic tail. As demonstrated by chemical cross-linking and in vitro transfection analyses, the 4 major UPs form 2 distinct pairs (UPIa with II and UPIb with IIIa) for them to exit efficiently from the endoplasmic reticulum during biosynthesis [16,17]. Because UPs are highly conserved during mammalian evolution [12], it has been suggested that they play key roles in urothelial functions, including participation in the permeability barrier, adjustment of urothelial surface area, stabilization of the urothelial surface, and development of the urinary tract [12,18,19]. Consistent with their proposed functions at the urothelial surface, UPs are expressed in a tightly differentiation-dependent manner, being detected ultrastructurally (in the form of AUMs) and immunohistochemically at the apical surface and cytoplasm of the superficial cell layer of human urothelium with little or no detection in the intermediate and basal layers [20,21].

The fact that UPs are the major differentiation products of urothelium strongly implies that UPs would be significantly downregulated during urothelial transformation and tumorigenesis. This was proven to be the case in cultured normal urothelial cells, where incomplete differentiation and/or hyperproliferation led to a profound reduction of all UPs, particularly on a protein level [22]. Similarly, in a number of human bladder cancer cell lines, UPs are either downregulated (RT4, RT112) or undetectable (J82, T24) [23] (unpublished observation). These results suggest that, like many epithelial differentiation markers, UP expression is profoundly perturbed and/or downregulated during urothelial transformation. Surprisingly, however, this idea is not supported by data from human urothelial carcinomas. For example, Moll et al showed by immunohistochemical staining that more than half of the invasive and metastatic urothelial carcinomas continued to express UPs [20]. Their results, combined with those of the subsequent studies from several independent groups [21,24], strongly suggest that UP downregulation does not strictly parallel urothelial tumorigenesis and progression. Most of the existing studies, however, assessed a relatively small number of urothelial carcinomas, with no correlation with clinical outcome. Key questions remain as to whether the status of UP is associated with a particular pathologic parameter, the carcinoma's biological behavior, or clinical outcome, and whether UPs can be useful markers for predicting patient prognoses.

Taking advantage of the recent availability of a set of tissue microarrays, we evaluated UP expression in a large cohort consisting of 335 bladder specimens from 285 patients that represent all stages and grades of urothelial carcinomas and different clinical outcome. This allowed us to correlate and systematically analyze the status of UP expression with pathologic parameters as well as disease prognoses. Our data indicate that UPs are retained by a significant portion of well-advanced urothelial carcinomas and that persistent UP expression in invasive and metastatic urothelial carcinomas