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Review article

When urothelial differentiation pathways go wrong: Implications for bladder cancer development and progression

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Abstract

Differentiation is defined as the ability of a cell to acquire full functional behavior. For instance, the function of bladder urothelium is to act as a barrier to the diffusion of solutes into or out of the urine after excretion by the kidney. The urothelium also serves to protect the detrusor muscle from toxins present in stored urine. A major event in the initiation and progression of bladder cancer is loss of urothelial differentiation. This is important because less differentiated urothelial tumors (higher histologic tumor grade) are typically associated with increased biologic and clinical aggressiveness. The differentiation status of urothelial carcinomas can be assessed by histopathologic examination and is reflected in the assignment of a histologic grade (low-grade or high-grade). Although typically limited to morphologic evaluation in most routine diagnostic practices, tumor grade can also be assessed using biochemical markers. Indeed, current pathological analysis of tumor specimens is increasingly reliant on molecular phenotyping. Thus, high priorities for bladder cancer research include identification of (1) biomarkers that will enable the identification of high grade T1 tumors that pose the most threat and require the most aggressive treatment; (2) biomarkers that predict the likelihood that a low grade, American Joint Committee on Cancer stage pTa bladder tumor will progress into an invasive carcinoma with metastatic potential; (3) biomarkers that indicate which pTa tumors are most likely to recur, thus enabling clinicians to prospectively identify patients who require aggressive treatment; and (4) how these markers might contribute to biological processes that underlie tumor progression and metastasis, potentially through loss of terminal differentiation. This review will discuss the proteins associated with urothelial cell differentiation, with a focus on those implicated in bladder cancer, and other proteins that may be involved in neoplastic progression. It is hoped that ongoing discoveries associated with the study of these differentiation-promoting proteins can be translated into the clinic to positively impact patient care.

Keywords: Bladder cancer; Transcription factors; Differentiation; FOXA1; Retinoic acid; PPARγ

1. Introduction

During development, cells undergo a series of genetic and epigenetic changes whereby they acquire specific characteristics that enable them to fulfill specialized functions. In response to insult or injury this differentiation process may be reversed to enable cells and tissues to undergo repair, through proliferation and other processes, before re-acquisition of their differentiated characteristics. In neoplasia, however, this process potentially fails to occur normally, leading ultimately to uncontrolled growth and tumor formation. Thus, cancer can be viewed, in part, as a defect in differentiation. In this review, we consider urothelial differentiation as a framework for evaluating molecular regulators implicated in the pathogenesis of urothelial cancer, and consider how such information could be used clinically as biomarkers of outcome, or as therapeutic targets.

2. Differentiation characteristics of normal urothelium

The urothelium that lines the bladder is a pseudostratified epithelium comprising discrete populations of epithelial cells that can be identified as basal, intermediate or superficial based on their localization relative to the basement

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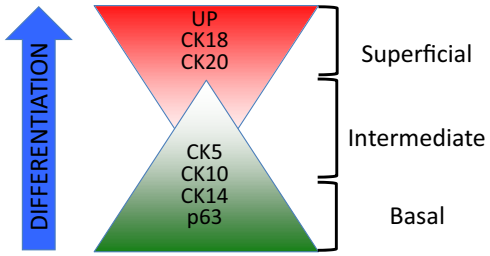

Fig. 1. Normal bladder urothelium displays a spectrum of protein markers identifying subpopulations of specific cell types. Although the pathways required for urothelial differentiation are not completely understood, it is theorized that differentiation of cytokeratin (CK) 14 and CK10 positive basal cells results in an intermediate, CK5 positive cell population. Presence of uroplakin (UP) and cytokeratin 18/20 positive cells, as well as absence of p63 expression, indicate the presence of superficial "umbrella cells," which are often lost early in bladder cancer progression. (Color version of figure is available online.)

membrane or bladder lumen, as well as expression of specific marker proteins (Fig. 1). Although this topic has been covered in many excellent articles [1,2], several points are germane to the discussion here. The urothelium is specified during embryonic development in response to inductive signals from the developing mesenchyme, and is characterized by a highly specialized superstructure on its luminal aspect termed the asymmetric unit membrane (AUM) [3–5]. The AUM results from the apical presentation of a family of uroplakin (UP) proteins on the most superficial 'umbrella' cell layer [6–8] that assemble into plaque-like structures, and serves as 1 component of a barrier to urine [9,10]. UP expression is diminished in intermediate cells and largely absent in basal cells, whereas expression of other markers such as p63 and selected cytokeratins is increased, in line with the less differentiated nature of these populations. Thus, one can envision a spectrum of differentiation marker expression, with a specific complement of genes/proteins corresponding to discrete cell populations with specific fates. It is generally accepted that differentiation of cells within the urothelium follows a pattern similar to that in other epithelia such as the gut and skin, where cells in the basal compartment that are thought to harbor the progenitor population mature into progressively more differentiated intermediate cells and ultimately terminally differentiated superficial cells. However, several recent studies suggest that basal/intermediate and superficial cells derive from distinct lineages [11,12].

3. Urothelial differentiation as a paradigm for identifying factors implicated in urothelial cancer development

As described by Hanahan and Weinberg, tumor cells display a number of characteristic 'hallmarks', including self-sufficient growth, unlimited replicative potential, and resistance to growth inhibitory signals among others [13]. In contrast, fully differentiated cells, such as those in the normal urothelium, display limited growth potential. Thus, if cellular differentiation is defined as the attainment of full functional capacity, cancer can be defined, in part, by loss of the differentiated phenotype. For example, in addition to promoting tumor progression and metastatic dissemination, loss of differentiation in urothelial carcinoma is accompanied by diminished barrier function. Conversely, alteration of the urothelial barrier following augmentation cystoplasty has been linked to increased susceptibility to neoplastic transformation [14–16], highlighting the intimate relationship between loss of differentiation and development of cancer.

Based on its diverse clinical presentation, as well as extensive molecular genetics analysis, it is now accepted that bladder cancer develops along a number of discrete pathways that display characteristic chromosomal alterations (reviewed in [17]). Broadly speaking, lesions can be classified into (1) well differentiated, non-invasive papillary cancers characterized by deletions in chromosome 9; (2) poorly differentiated muscle-invasive tumors that show alterations in canonical tumor suppressors and oncogenes including p53, Rb, and PTEN; and (3) a distinct entity, carcinoma in situ (CIS) that, although confined to the urothelium, exists as a flat, non-papillary, poorly differentiated lesion displaying genetic alterations characteristic of both papillary and muscle-invasive tumors [18] Importantly, the presence of CIS correlates strongly with the risk of invasive disease, demonstrating the close association between loss of urothelial differentiation and ultimate development of aggressive bladder cancer.

The differentiation state of cells is a function of how cells respond and communicate with the local tissue milieu, and communication between stromal/mesenchymal and epithelial tissue compartments is an essential event for both the normal development of organs, as well as their maintenance [14,19–23]. While bladder mesenchyme provides signals that program the target epithelium to maintain a urothelial phenotype, epithelium must be receptive to these signals for proper differentiation. In addition, urothelial cells also send reciprocal signals to promote terminal differentiation of the bladder mesenchyme [24]. Cells utilize a number of mechanisms to respond to their microenvironment. For example, transcription factors function to integrate incoming signals from activated cell surface receptors, enabling cells to respond to microenvironmental stimuli. Thus, cell surface receptors and transcription factors represent excellent candidate biomarkers for (1) the extent of urothelial differentiation, (2) cancer diagnosis and/or prognosis, and (3) identification of groups of deregulated genes which could serve as novel targets for personalized therapy.

4. Histopathology of urothelial neoplasia and utility of existing biomarkers

By definition, increased urothelial cell growth is accompanied by a loss of differentiation. Classification of malig-