CD24 expression is important in male urothelial tumorigenesis and metastasis in mice and is androgen regulated

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AUTHOR SUMMARY

Bladder cancer is the most common cancer affecting the urinary system and is one of the most expensive to treat. In the United States, nearly 73,510 new cases and 14,880 deaths are expected in 2012 (1). Bladder cancer may cause both noninvasive (superficial) and invasive tumors (2). The invasive type heralds tumor spreading to other body parts (metastasis) in nearly half of diagnosed patients and mostly is incurable by current therapies. The incidence of bladder cancer in the United States is three times higher in men than in women. Importantly, this difference persists even when differences in exposure to risk factors, such as smoking, are considered (3). The reasons for this discrepancy remain unclear. Here we investigated the role of one particular cancer-associated protein, CD24, and found that its absence reduces the incidence of bladder cancer and metastasis in male mice and is associated with better prognosis in male patients.

CD24 is a protein located on the surface of cells (4) and is rarely expressed in normal tissue (4), but it is found in a wide range of cancers and has significance in predicting disease outcome (prognosis) (5). In models of bladder cancer, loss of CD24 function is associated with decreased cell proliferation and reduced lung metastasis (5). Evaluation of CD24, using specific antibodies to semi-quantify a specific protein, revealed that increased CD24 expression on human bladder tumors correlated with decreased disease-free survival (5).

Despite a significant body of work in many cancer types, the distinct requirement for CD24 in the growth of tumors and spontaneous metastasis remains uncharacterized. Because cancer-causing agents [e.g., carcinogens such as cigarette smoke (3)] are the primary risk factors for bladder cancer, we used a carcinogenesis-based animal model to address this gap in knowledge. We induced invasive and metastatic bladder cancer by using the common carcinogen N-butyl-N-(4-hydroxybutyl) nitrosamine. Strikingly, we found that WT male mice had a higher incidence of bladder cancer tumor formation than WT female mice (Fig. P1); this finding is consistent with bladder cancer being three times higher in men than women. Even more intriguing was the discovery that this increased incidence in male mice was reversed in male CD24a-knockout mice (CD24a−/−).

Fig. P1. The loss of the Cd24a gene in mice reduces tumor formation in a sex-specific manner. Comparison of tumor incidence over time revealed that male WT mice had a significantly higher incidence of tumor formation than female mice. Interestingly, Cd24a deficiency in males dramatically reduced this incidence in males but had no impact in female mice. The studies presented here suggest that this difference in tumor formation in the sexes is driven by androgen. This male-specific hormone is shown to increase directly expression of CD24, a known tumor-promoting protein. Thus, the presence of androgen in male mice leads to an increase in CD24 and an increase in tumor formation. However, the ability of androgen to induce tumor formation is reduced dramatically if CD24 is eliminated (Cd24a−/−). AR, androgen receptor.

Given the sex-dependent role of CD24, we investigated a possible relationship between CD24 and the male hormone androgen. Like other hormones, androgen binds to a receptor and initiates a series of events to alter cell function. We found that depletion of the androgen receptor from UM-UC-3 human carcinoma cells reduced both CD24 levels and cell proliferation. Furthermore, cellular proliferation also was reduced following depletion of CD24, suggesting that CD24 and androgen receptor function together to help regulate cell growth. Such an observation, coupled with the findings described above that loss of CD24a in mice could inhibit the male-specific propensity for tumorigenesis, suggests that CD24 functions as a downstream effector of androgen signaling. In fact, additional analysis determined a specific sequence within the promoter of the CD24 gene which associates with androgen receptor. Thus, our studies strongly suggest that androgen/androgen receptor complexes bind the CD24 promoter and promote an increase in CD24 expression (Fig. P1).

To determine if this mechanism translates to an in vivo model, the CD24 protein levels in UM-UC-3 human cells transplanted into the flanks of mice (xenografts) were investigated in normal male mice and in male mice that had been castrated (thereby inducing a loss of androgen). We found that the CD24 protein levels of these xenografts were lower in castrated mice than in controls. Furthermore, this loss


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Data deposition: The sequences reported in this paper can be found in GenBank database [JN656036 (UM-UC-3 CD24 promoter)]; JN655037 (J82 CD24 promoter); JN655040 (LNCap CD24 promoter); JN565041 (Lu-2 CD24 promoter); and JN565042 (EJ CD24 promoter).

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of androgen, and therefore of CD24, led to reduced tumor growth. Interestingly, this growth reduction was mitigated when UM-UC-3 cells were engineered to express CD24 independently of androgen. Thus, our findings suggest that androgen-dependent CD24 expression is a downstream regulator of bladder cancer growth and metastasis in males.

Interestingly, while analyzing the metastatic spread of cancer, we found that CD24 also plays a sex-specific role. In mice with evidence of primary bladder cancer (using histopathology, or microscope analysis), we found that Cd24a-deficient male mice had fewer metastases than their WT counterparts. Additionally, male patients with bladder cancers who had the highest levels of CD24 expression had the poorest prognosis for disease-free survival. This trend was not seen in female patients.

In conclusion, our study shows that CD24 has an important role in sex-specific bladder tumor formation and metastasis in a mouse carcinogenesis model. These studies also show that CD24 is regulated by androgens and may be an important molecule responsible for androgen-mediated signaling pathways in this and other cancers. Finally, given the role of CD24 in tumor initiation and progression in bladder cancer, and its regulation by androgen, therapies directed at the androgen receptor may be of benefit in men with bladder cancer. Such an approach would indicate that targeting androgen-dependent signaling pathways in males might be important for controlling or preventing cancers other than prostate cancer.