DIFFERENTIAL EXPRESSION OF C-CAM CELL ADHESION MOLECULE IN PROSTATE CARCINOGENESIS IN A TRANSGENIC MOUSE MODEL

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ABSTRACT

Purpose: The transgenic adenocarcinoma of mouse prostate (TRAMP) model, in which various grades of prostate intraepithelial neoplasia (PIN) and prostate cancer with metastases can be reproducibly generated, is a paradigm for prostate disease progression. We have previously shown that C-CAM, an adhesion molecule, can suppress the growth of prostate cancer. In this report, we describe immunohistochemical characterization of differential expression of C-CAM at various stages of prostate tumorigenesis in the TRAMP model.

Materials and Methods: We sampled prostate specimens and periaortic lymph nodes from TRAMP mice. Indirect immunohistochemical staining with a polyclonal anti-C-CAM antibody was performed on the formalin-fixed, paraffin-embedded specimens. After castration at 12 weeks of age, the TRAMP mice developed androgen-independent prostate cancer (AIPC) and lymph node metastasis at 18 to 24 weeks of age. Samples from these castrated mice were also analyzed.

Results: C-CAM protein was expressed in the normal prostate epithelia of non-transgenic and TRAMP mice as well as in low-grade PINs in TRAMP mice. Expression was uniform on the luminal surfaces of these epithelia. C-CAM expression was noticeably reduced and the staining pattern heterogeneous in some high-grade PINs. C-CAM staining was generally absent in prostate cancer and metastatic lymph nodes. Androgen independent prostate cancer and its metastatic tumors generated in castrated TRAMP mice were also C-CAM negative.

Conclusions: C-CAM expression correlates with the differentiation states of prostate epithelia and is down regulated early in prostate tumorigenesis in the TRAMP model.

Key Words: cell adhesion molecule, transgenic mice, prostatic neoplasms

Prostate cancer is the most common non-skin cancer and the second leading cause of cancer death in American men.1 Like other forms of cancer, prostate cancer is believed to be a multistep disease and is associated with cumulative genetic alterations in prostatic epithelial cells. Identification of molecular markers that are differentially expressed or altered in prostate tumorigenesis may help us design more effective treatment and prevention strategy for prostate cancer.

Cell adhesion molecules (CAMs) are cell-surface glycoproteins that mediate cell-to-cell or cell-to-extracellular matrix adhesion. Studies on the functions of CAMs have shown that they play important roles in cellular development, morphology and motility.2 CAMs may also mediate signal transduction that are important for cell growth and differentiation.3,4 C-CAM is an epithelial CAM originally identified by Ocklind and Obrink in 1982 by its ability to mediate hepatocyte aggregation.5 Moderate to high levels of C-CAM message are detected in liver, intestines, lung, parotid gland, and renal tubules.6,7 In rat prostate, C-CAM is primarily expressed in the glandular epithelial cells, and its expression is regulated by androgen.8 C-CAM expression levels are significantly lower in hepatomas9 and colon cancer cells10–12 than in their benign counterparts. Thus, C-CAM seems to be expressed mostly in well-differentiated epithelia, and its expression is down regulated in less-differentiated cells such as cancer cells. These observations suggest that C-CAM may play an important role in maintaining cellular differentiation. Prostate cancer, like other cancers, develops in a multistep process that leads to aberrant protein expression. One good way to define the role of C-CAM in prostate cancer is to assess the changes in its expression at various stages during prostate cancer progression.

The absence of animal models for autochthonous spontaneous prostate cancer has been a major obstacle in the study of prostate tumorigenesis and the design of new therapeutic strategies. Transgenic mice that are genetically modified to confer susceptibility to neoplastic growth of the prostate epithelia are valuable models for better understanding prostate tumorigenesis. A system utilizing the rat probasin promoter to target simian virus 40 large T antigen specifically to mouse prostate13 has been successfully used to generate a transgenic adenocarcinoma mouse prostate (TRAMP) model.13,14 In this TRAMP model, the probasin-T antigen transgene is specifically expressed in the dorsolateral and ventral prostate when mice reach sexual maturity at 5 to 7 weeks of age. By 8 weeks of age, T antigen oncoprotein is readily detectable in the dorsolateral prostate. Mild to severe hyperplasia of dorsolateral prostatic epithelia can be observed as early as 10 weeks of age. More importantly, TRAMP mice reproducibly develop PIN and prostate cancer as early as 10 to 12 weeks of age. By 18 weeks, invasive prostate adenocarcinoma can be seen in nearly all TRAMP mice, and metastases can be detected as early as 12 weeks. By 30 to 36 weeks, nearly all TRAMP mice develop not only primary prostatic tumors but also metastases in lymph nodes, lungs and, occasionally, bone. A recent longitudinal cohort analysis of lethal prostate cancer progression in TRAMP mice showed that palpable tumors always preceded lethal pro-
C CAM EXPRESSION IN TRAMP MODEL 893

MATERIALS AND METHODS

Transgenic animals. Male and female TRAMP mice, heterozygous for the probasin-T antigen transgene, were maintained on a pure C57BL/6 background (Harlan Sprague Dawley, Inc., Indianapolis, IN). Transgenic males for these studies were routinely obtained by (TRAMP X C57BL/6) or (TRAMP X FVB Breeder) crosses. Isolation of mouse-tail DNA and polymerase chain reaction-based screening assays were performed as described previously. Non-transgenic normal adult male mice served as the controls. The TRAMP mice were sacrificed between 12 and 20 weeks of age and prostate and periaortic lymph nodes harvested. To generate androgen-independent tumors, TRAMP mice were castrated at 12 weeks of age and sacrificed at 18 to 24 weeks of age. All experiments were performed with the highest standards for humane care of animals in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Tissue preparation. At necropsy, the lower urinary tract, including the bladder, proximal urethra, seminal vesicles and prostate, were removed en bloc. Periaortic lymph nodes were also sampled for histological examination. Tissues collected at necropsy were fixed in 10% (v/v) phosphate-buffered formalin for 6 hours and then treated with 70% ethanol before standard tissue processing. Sections (4 μm) were cut from the paraffin-embedded tissues and mounted on ProbeOn-Plus slides (Fisher Scientific, Houston, TX). The various grades of PIN and prostate cancer were defined histologically as previously stated.

Antibody against C CAM. We used polyclonal rabbit antibody Ab669 which was generated against the full-length rat C CAM. This antibody has excellent cross-reactivity with mouse C CAM, which is 71% similar to rat C CAM.

Immunohistochemical staining of C CAM protein. Tissue sections from formalin-fixed, paraffin-embedded specimens were dewaxed with xylene and rehydrated in graded alcohol. These sections were then treated with 3% H2O2 in methanol at room temperature for 15 minutes, washed with phosphate-buffered saline (PBS), blocked with normal goat serum at room temperature for 30 minutes, and then incubated at room temperature for 1 hour with Ab669 anti-C CAM antibody (diluted 1:2,000 in 0.1% bovine serum albumin in PBS). After two washes with PBS, biotinylated goat anti-rabbit secondary antibody was added and incubated at room temperature for 30 minutes. The StrAviGen Super Sensitive streptavidin conjugated with horseradish peroxidase detection system (Biogenex Laboratories, San Ramon, CA) was used according to the manufacturer's instructions with 3,3'-diaminobenzidine as the chromogen. The immunostained sections were then counterstained with hematoxylin. A section from each block was stained with hematoxylin and eosin (H & E) to determine the histopathology.

RESULTS

By immunohistochemical staining with Ab669 polyclonal antibody, C CAM expression was localized to the luminal surface of dorsolateral and ventral prostate epithelia of normal adult male mice (fig. 1, A). No C CAM staining was detectable at the cell junctions or the cell borders with the basement membrane (fig. 1, A). In TRAMP mice, C CAM expression was detectable in the normal prostate epithelia of sexually mature mice (6 to 8 weeks of age) (fig. 1, B). In low-grade PINs in the TRAMP mice, C CAM was also localized to the luminal surface of atypical epithelia, and the level of C CAM expression was similar to that observed in normal acini (fig. 1, C and D). In these low-grade PIN samples, mitosis and apoptotic bodies were commonly found, and normal-looking epithelia cells and low-grade atypical cells were present in the same acinus (fig. 1, C and D). In high-grade PINs, C CAM staining was reduced and became heterogeneous, such that some staining was still detected on the luminal surface of atypical cells (fig. 1, E) but was absent in proliferative cell masses that did not form a glandular structure or border a glandular lumen (fig. 1, F). This staining pattern indicates that C CAM expression was down regulated in atypical cells that had become proliferative and dedifferentiated.

C CAM staining was generally undetectable in primary prostatic tumors from the dorsolateral prostates of the TRAMP mice where, as early as 10 to 12 weeks of age, we found low-grade tumors that appeared as multiple closely packed microacinar structures without intervening connective tissue (fig. 2, A). The positive C CAM staining in low-grade PINs around tumors presented a good contrast to the negative staining of the low-grade tumor nests (fig. 2, B). C CAM expression was also absent in high-grade prostate tumors, which appeared as sheets or nests of poorly differentiated tumor cells with irregular nuclei and prominent nucleoli (fig. 2, C). Occasionally, a few tumor cells that formed immature abortive glandular structures within C CAM-negative high-grade tumor background had weak C CAM staining (data not shown). This observation suggests that C CAM expression may be closely linked to cellular differentiation: the more differentiated the cells, the higher the C CAM expression. Metastatic deposits in periaortic lymph nodes could be found as early as 12 weeks of age in the TRAMP mice. These hyperchromatic poorly differentiated tumor cells in the lymph nodes did not show C CAM staining (fig. 2, D).

Interestingly, C CAM staining of normal prostatic acini in both the dorsolateral and ventral prostate seemed to be stronger in the castrated mice than in the intact ones (fig. 3, A). By 18 weeks of age, androgen-independent primary prostate tumors could be seen in some TRAMP mice castrated at 12 weeks of age. These androgen-independent tumor cells were generally poorly differentiated and negative for C CAM staining. In few areas where small immature glandular patterns formed in sheets of poorly differentiated tumor cells, weak C CAM staining could be observed on the luminal cell surfaces (fig. 3, B). Metastatic deposits from the androgen-
FIG. 1. C-CAM expression in prostate glands of intact control and TRAMP mice. A, normal secretory acinus of dorsolateral prostate (non-transgenic control mouse, 12 weeks old, ×400). C-CAM staining (yellowish brown color) is present on luminal surface of secretory epithelia. Nuclei are homogeneous and round to ovoid. Nucleus to cytoplasm ratio (N/C ratio) is low. Cells are loosely packed. Mitosis is rarely seen. B, normal-looking acinus (TRAMP mouse, 12 weeks old, ×400). C-CAM staining is positive, and histologically acinus does not differ from that of non-transgenic mouse. C, low-grade PIN (TRAMP mouse, 12 weeks old, ×400). C-CAM staining is present on luminal surface. Cells are more closely packed than those of normal acini. Nuclei are strongly stained and elongated. N/C ratio is higher than normal. Array of cells (arrow) is still normal-looking. Apoptotic body can be seen in this acinus (arrowhead). D, low-grade PIN (TRAMP mouse, 12 weeks old, ×400). C-CAM staining is positive. Acini have strongly stained, closely packed nuclei. There are few normal-looking cells present in atypical gland (arrow). E, high-grade PIN (TRAMP mouse, 12 weeks old, ×400). C-CAM expression is generally present on multiple luminal surfaces of this cribriform-like acinus. Nuclei are heterogeneous in size and have high N/C ratio. Basement membrane is still intact. F, high-grade PIN in a larger acinus (TRAMP mouse, 12 weeks old, ×400). Acinus appears to be growing rapidly and has cribriform growth pattern. C-CAM is absent in those preneoplastic cells that do not form glandular lumen (arrow).
independent primary cancer could be readily detected in periaortic lymph nodes, and those deposits were also devoid of C-CAM staining (fig. 3, C).

**DISCUSSION**

In this study, we observed that C-CAM expression was down regulated at a relatively early stage in prostate tumorigenesis in the well-characterized TRAMP model and the degree of this down regulation seemed to correlate with advancing degree of tumorigenesis. These observations lead to two possible conclusions with respect to the role of C-CAM in prostate tumor progression. In the first scenario, loss of C-CAM may simply result from loss of glandular lumens and therefore was indirectly associated with malignant process. C-CAM was found to express on the apical surface of luminal epithelial cells and the morphology of glandular lumens disappeared as tumors progressed. As a result, decrease in C-CAM expression may be a secondary event associated with dedifferentiation of luminal epithelial cells. If so, C-CAM may not be actively involved in the malignant process. On the other hand, it is also possible that decreased C-CAM expression is directly involved in the dedifferentiation of the luminal epithelial cells that lead to malignant progression. Since the histochemical studies as described here cannot distinguish between these two possibilities, one can only conclude that inactivation of C-CAM is one of the events associated with malignant transformation.

Whether it is due to primary or secondary event, correlation of C-CAM expression with the differentiation stages of the cells is evident from our immunohistochemistry studies. C-CAM expression was readily detectable in well-differentiated normal prostate glandular epithelium and in low-grade PINs. However, C-CAM expression was lower in high grade PINs where preneoplastic epithelia had become highly dedifferentiated. C-CAM was not expressed in primary and metastatic prostate cancers or in androgen-independent tumors or metastatic deposits in castrated TRAMP mice. The observation that C-CAM expression was reduced in high-grade PINs and was absent in prostate cancer suggests that C-CAM might be a unique marker for early prostate tumorigenesis.

It is interesting that in poorly differentiated and C-CAM negative cancer masses, weak C-CAM staining appeared on the luminal surface of some cancer cells that formed immature glandular structures. Tumors are often composed of heterogeneous populations of cells with differing degree of differentiation. Thus, even in high grade tumors, there might be some differentiated cells that form the glandular structure. This and the expression of C-CAM in benign differentiated prostate epithelia but not in cancerous cell masses

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**FIG. 2. Loss of C-CAM expression in prostate tumors of TRAMP mice.**

**A.** Low-grade primary prostate tumor (12-week-old mouse, ×100). It has multiple closely packed and regular glandular structures without intervening connective tissue. Pseudo-capsule outlines tumor nodule (arrow) which appears to be C-CAM negative. Area with low-grade PINs can be seen to left of tumor. **B.** Close-up view of tumor in panel A. C-CAM staining is positive in low-grade PINs, in contrast to negative staining in tumor nodule (200X). **C.** High-grade primary tumor (18-week-old mouse, ×400). There is sheet of C-CAM-negative, poorly differentiated tumor cells with irregular nuclei. Invasion of periprostatic soft tissue is evident. **D.** Periaortic lymph node metastasis (18-week-old mouse, ×400). Metastatic tumor cells (tumor) occupy right lower part of field and are encroaching on normal lymphoid tissue (lymph). Tumor thrombus can be seen in perinodal blood vessel (arrow). C-CAM expression is negative in these tumor cells.
suggest that C-CAM expression may correlate with cellular differentiation. As cells became dedifferentiated, C-CAM expression was downregulated. Therefore, C-CAM may serve as a differentiation marker for prostate epithelium.

In summary, the TRAMP model is a good paradigm for studying prostate tumorigenesis. C-CAM was downregulated early in prostate tumorigenesis in the TRAMP model and C-CAM expression correlated with the differentiation stages of prostate epithelia. These observations support our hypothesis that loss of C-CAM is an early event in prostate tumorigenesis and that C-CAM may be a useful marker for the diagnosis and prognosis of prostate cancer.

REFERENCES