Consistent Expression of an Epithelial Cell Adhesion Molecule (C-CAM) during Human Prostate Development and Loss of Expression in Prostate Cancer: Implication as a Tumor Suppressor

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Abstract

Cell adhesion molecules have been suggested to function as tumor suppressor molecules. We have been studying one of the epithelial cell adhesion molecules (C-CAM), which belongs to the immunoglobulin gene superfamily. Transfection of a C-CAM cDNA expression vector into a highly tumorigenic human prostate cancer cell line (PC-3) suppresses tumor formation in nude mice. Alternatively, reducing C-CAM expression levels in the nontumorigenic rat prostate epithelial cell line NbE by the antisense expression vector markedly increases tumorigenicity of NbE cells in nude mice. These results suggest that C-CAM may be a tumor suppressor in prostate cancer.

In this study, we examined the relationship between C-CAM expression during human prostate development and neoplastic progression by immunohistochemical staining of frozen sections. C-CAM predominantly localized on the plasma membrane of the basal cell layer in both the fetal and normal adult prostate gland. However, an overall decreased staining was seen in benign prostatic hyperplasia and high grade prostatic intraepithelial neoplasia. Furthermore, C-CAM was not detected in prostate carcinomas. Thus, a decrease in C-CAM expression may be an early event in hyperplastic/neoplastic transformation. These observations support the suggestion that C-CAM is a tumor suppressor in prostate cancer progression.

Introduction

Malignant transformation is associated with an alteration in normal cellular differentiation. During the development of a multicellular organism, CAMs are known to play a critical role in maintaining the differentiated status of epithelial cells. Recent studies in several neoplasms suggest that CAMs may be tumor suppressors (1–4). Although the underlying functional mechanisms associated with CAMs remain undefined, it is likely that CAMs transduce their signals to cells through interactions with other regulatory molecules. For example, the E-cadherin-associated proteins (i.e., α- and β-catenin) are capable of interacting with a potential tumor suppressor gene product, i.e., APC (5, 6).

C-CAM was originally isolated from the plasma membrane of the rat hepatocyte by its reaction with specific antibodies that neutralize cell aggregation (7). Recent studies indicate that C-CAM, with an apparent molecular weight of 105,000, is expressed in virtually all epithelial cells (8). Structurally, C-CAM belongs to the immunoglobulin superfamily and its sequence is highly homologous to carcinoembryonic antigen (9). Using a baculovirus expression system, Cheung et al. (10) demonstrated that the first immunoglobulin domain of C-CAM is critical for cell-adhesive activity. Decreased C-CAM levels have been associated with several neoplasms including hepatoma and colorectal carcinoma (4, 11), suggesting that it may also play a suppressive role in tumorigenesis.

Although little is known about the role of C-CAM in prostate cancer progression, we have shown that its expression in the prostatic epithelium can be regulated by androgen in an organ-specific manner (12). Furthermore, the pattern of C-CAM expression correlated with androgen-induced prostatic epithelial differentiation (12), suggesting that C-CAM acts as a homeostatic signal during this process. To further examine this hypothesis, we demonstrated that transfection of C-CAM into an androgen-independent human prostate cancer line (PC-3) that does not express C-CAM resulted in a diminished tumorigenicity when inoculated s.c. into athymic nude mice (1). Conversely, down-regulation of C-CAM levels by antisense C-CAM gene transfection in a nontumorigenic rat prostate epithelial cell line (13) with detectable C-CAM, NbE, markedly increased tumorigenicity in vivo (1). On the basis of these observations, we decided to investigate C-CAM expression during both prostate development and cancer progression. Using immunohistochemical staining for C-CAM, we observed a typical plasma membrane staining pattern in the basal cell layer of the normal prostate gland obtained from fetal, juvenile, and adult prostates. In contrast, we detected a complete disappearance of C-CAM in prostate carcinoma. These results suggested that down-regulation of C-CAM may be associated with the onset of hyperplastic/neoplastic transformation.

Materials and Methods

Immunohistochemistry. Tissue samples from radical prostatectomy (25 patients) and transurethral resection of the prostate (1 patient) were obtained from the Department of Pathology at the M. D. Anderson Cancer Center. The tissue samples were immediately placed in tissue blocks with OCT compound (Miles, Elkhart, IN) and frozen at −20°C. Both prostate carcinomas and normal prostatic tissues were included in this study. Serial 4-μm-thick sections were cut from each sample with a cryostat. One section was stained with hematoxylin and eosin for pathological evaluation and grading; adjacent sections were used for immunohistochemical staining. The prostate adenocarcinomas were graded according to the Gleason grading system. PIN was classified as low grade (PIN1) and high grade (PIN2 and PIN3) as agreed at the 1989 Workshop on Prostatic Dysplasia (14). In addition, prostate samples representative of prostate gland development, such as fetal prostates (30 and 36 weeks old) and a prostate from a 13-year-old boy were included in the study. These samples were provided as frozen sections by Sherwood et al. (15).

For immunoperoxidase staining, tissue was placed on silane-coated slides, which were then rinsed in PBS. The tissue was fixed in a solution of 95% methanol and 5% acetic acid at −20°C for 15 min. Endogenous peroxidase...
activity was blocked with 3% hydrogen peroxide in methanol at room temperature for 15 min. After the tissue was washed with PBS three times for 5 min each, Superblock (Scytek Laboratories, Logan, UT) was applied for 20 min at room temperature. Slides were then incubated with a polyclonal antibody specific against C-CAM [Ab 669 (16), 1:2000 dilution in PBS containing 1% BSA] overnight at 4°C. The negative controls were incubated with preimmune serum. A biotin-avidin amplification system using a StrAvi-Gen Super Sensitive kit (Biogenex Laboratories, San Ramon, CA) with diaminobenzidine as the chromagen was applied. A light green dye (Sigma Chemical Co., St. Louis, MO) was used for background counterstaining.

Immunohistochemical staining was evaluated by three observers (D. I. K., P. T., and J. T. H.). The pattern of staining was classified as positive with a continuous staining (staining in a continuous pattern surrounding any given gland), positive with a discontinuous staining (staining in a broken or discontinuous pattern surrounding any given gland), or negative staining (no staining at all).

Results and Discussion

Expression of C-CAM during Human Prostate Development. The anlage of the human prostate originates during fetal development as solid buds arising from the urogenital sinus and the vesicourethral components of the cloaca. The bud stage (20–30 weeks of gestation) is characterized by solid epithelial buds at the ends of ducts without a recognizable lumen (17). Using cell type-specific cytokeratin antibodies for either basal or luminal cells, Sherwood et al. (15) clearly showed that only basal cells were present in these epithelial buds. By 36 weeks of gestation, some buds are still visible; however, the majority have developed into acinotubular structures with identifiable lumens (17).

To understand the expression pattern of C-CAM during prostate development, we studied the same 30- and 36-week-old fetal prostate specimens by immunohistochemical staining using a polyclonal antibody specific against C-CAM (i.e., Ab 669). Recent data indicated that Ab 669 raised against the rat C-CAM molecule can cross-react with the human counterpart, BGP-1 (biliary glycoprotein) (18). Also, this antibody appeared not to cross-react with carcinoembryonic antigen and other immunoglobulin-like CAMs (18). In the 30-week-old fetal prostate, as shown in Fig. 1A, C-CAM expression could be detected as typical plasma membrane staining associated with multiple basal cell layers in the epithelial buds. In contrast, the surrounding stromal compartment stained negatively (Fig. 1A). In addition to epithelial buds in the 36-week-old fetal prostate, there were acinotubular structures with identifiable lumens. C-CAM expression localized predominantly in the basal cell layers of these maturing glands (Fig. 1B). Luminal epithelium showed weak if any staining. Furthermore, tissue obtained from a 13-year-old prostate exhibited a continuous positive staining of C-CAM associated with the basal cell layer (Fig. 1C), and again the luminal epithelium showed weak if any staining. In contrast, the antibody controls showed a negative C-CAM staining (Fig. 1D). In the normal adult prostate gland (Fig. 2, A and

Fig. 1. Immunohistochemical staining of C-CAM protein in both fetal and juvenile human prostate. A, positive plasma membrane staining in multiple cell layers of a 30-week-old fetal prostatic bud (arrow). × 400. B, positive basal layer staining in a 36-week-old fetal prostatic tubule (arrow). × 400. C, positive basal layer staining in a 13-year-old prostate (arrow). × 400. D, preimmune serum staining from a 13-year-old prostate. × 200.
C-CAM expression showed the same positive staining pattern in the basal cell layer as was observed in the fetal prostates (Fig. 1). Taken together, these data indicate that C-CAM expression in the normal human prostate gland is associated with basal cells throughout the early fetal stage into adulthood. In contrast, the rat ventral prostate shows a different staining pattern; steady-state levels of C-CAM are detected in the luminal epithelium from intact animals; however, elevated levels of C-CAM are detected in the basal cell layer only after androgen deprivation (12). This difference in C-CAM staining pattern between the two species may be due to differences in the proliferative potential of luminal epithelium. For example, in the intact rat prostate, proliferative activity is found in the luminal
epithelium (19); however, in the human prostate, the majority of proliferative activity is confined to the basal cell population (20).

Expression of C-CAM in Basal Epithelium from Different Zones of the Normal Prostate Gland. Consistent with the results from fetal prostate development, we observed a continuous plasma membrane staining of C-CAM predominantly associated with basal cells in the normal glandular epithelium from the peripheral (Fig. 2, A and B), central (data not shown), and transition zones (Fig. 2, E and F) of the prostate. As summarized in Table 1A, we observed the same continuous staining pattern of C-CAM in the basal cell layer in all specimens examined (i.e., 29 cases) regardless of zonal origin. On the other hand, luminal cells showed weak if any staining. In addition, atrophic glands (17 cases) with a distinct basal cell layer, exhibited a continuous C-CAM staining pattern in the basal cell layer in any given gland (Table 1A).

Decreased Expression of C-CAM in BPH and PIN. BPH, often found in the transition zone/periurethral gland region of the human prostate (21), is considered a hyperplastic growth of epithelium and/or mesenchyme. In this study, an overall decrease in C-CAM staining was observed in the epithelial compartment from BPH glands compared with adjacent normal glands in the transition zone (Fig. 2, E and F). The overall C-CAM staining pattern of basal epithelium from BPH glands can be divided into two categories: (a) as shown in Table 1A, in 40% of specimens examined, the basal epithelium from BPH glands showed a continuous staining pattern of C-CAM with a similar staining to normal glands, but the majority (i.e., 60%) of specimens showed either a discontinuous staining pattern in the basal layer (Fig. 2E) or no staining at all (Fig. 2F). However, in the adjacent normal (nonhyperplastic) glands from the same tissue section, the basal cell layers exhibited a continuous C-CAM staining (Fig. 2, E and F). Therefore, decreased C-CAM expression may be associated with hyperplasia of the prostatic epithelium.

PIN is considered a precursor of prostate carcinoma (22, 23). Therefore, it is important to determine C-CAM expression in PIN. Our findings on PIN are limited to high-grade (PIN2 and PIN3) lesions that could be reproducibly identified on the frozen sections. Because low-grade PIN (PIN1) could not be unequivocally distinguished from epithelial hyperplasia or other minor epithelial atypias on frozen sections, it was not included in our evaluation. As summarized in Table 1A, the overall C-CAM-staining pattern associated with basal cells is altered in high grade PIN glands. For example, although some PIN glands still exhibited a continuous C-CAM staining pattern in the basal cell layer (Fig. 2C), adjacent PIN glands had a discontinuous pattern of C-CAM staining along the basal cell layer (Fig. 2C). Very often, this discontinuous pattern of C-CAM staining associated with PIN was seen adjacent to areas of carcinoma, which were completely negative for C-CAM staining (Fig. 3, A and B). Notably, we also observed a mixed pattern of both continuous and discontinuous staining associated with the basal layer within a single PIN gland (Fig. 2D). These observations suggested that the altered and decreased C-CAM expression may be associated with the continuous progression from normal prostatic epithelium to premalignant transformation and finally to carcinoma (24). These results suggest that altered C-CAM expression may be an early event associated with prostate cancer development.

Absent Expression of C-CAM in Primary Prostate Carcinomas. Prostate cancer is a multifocal disease with a wide range of histological patterns and biological potential. The heterogeneous staining pattern of C-CAM in premalignant PIN glands prompted us to examine the relationship of C-CAM expression in prostate cancers of different histological grade and zonal origin. In all 22 prostate carcinoma specimens examined, a complete absence of C-CAM staining was observed regardless of zonal origin (Fig. 3; Table 1B). The carcinomas studied were moderately and poorly differentiated tumors (Gleason score, 5–10). Although we did not examine well-differentiated carcinoma (Gleason score, 2–4), areas of histological grade 2 present focally among the moderately differentiated tumors were also negative. In contrast, normal glands from the same sections still retained a continuous staining pattern of C-CAM, indicating that the absence of staining in carcinoma was not due to staining artifact (Fig. 3, C and D). All preimmune serum controls were negative (data not shown). The complete loss of C-CAM even in the well-differentiated foci within moderately differentiated tumors suggests that the loss of C-CAM occurs early in malignant transformation.

Tumor Suppressive Role of C-CAM in Prostate Cancers and Other Neoplasms. Consistent with the absence of C-CAM expression in human prostate cancer specimens, C-CAM was also not detected in several established human prostate cancer cell lines, including PC-3 (1) and DU-145 and LNCaP cells.4 Despite this inverse relationship between C-CAM expression and the presence of prostate cancer, little is known about the functional role of C-CAM in the development of prostate cancer. Recent data from our laboratory demonstrated that C-CAM plays a tumor-suppressive role in prostate cancer progression (1). Also, the down-regulation of C-CAM is consistently associated with many neoplasms. For example, diminished or decreased expression of C-CAM is observed in both primary and transplantable rat hepatocellular carcinoma (11). In human colorectal carcinoma, the expression of BGP-1, the human counterpart of C-CAM, is diminished (4). The mouse homologue, mouse biliary glycoprotein, is diminished through down-regulation at both the transcriptional and post-transcriptional levels in mouse colon tumors (25). Thus, the loss of C-CAM expression may be due to mechanisms other than gene deletion. In any case, these observations suggest that C-CAM could be a tumor suppressor in many epithelial malignancies.

Interestingly, data from other investigators (26, 27) showed that down-regulation of E-cadherin, a Ca2+-dependent CAM, is associated with both rat and human prostate cancer. These data further suggest that CAMs indeed play an important role in prostate cancer progression. However, E-cadherin staining in prostate cancer cells (26, 27)
conceivable that alteration of homeostatic signals in basal cells may determine the growth capacity of the prostate gland, and it is the presence of putative homeostatic signals in basal (or stem) cells that may have a different function than C-CAM in prostate cancer development, because these CAMs may possess unique signal pathways. For example, E-cadherin-associated proteins (i.e., α- and β-catenin) have been found to interact with another potential tumor suppressor gene product, i.e., APC (5, 6). Therefore, further exploration of C-CAM-associated molecules in human prostate cancer is warranted.

Potential Role of C-CAM in Prostate Gland Homeostasis. Maintenance of the prostatic epithelium is dependent on androgen; androgen deprivation can induce apoptosis in luminal prostatic epithelium, leading to degeneration of the prostate gland. In contrast, the basal cells are more resistant to the apoptotic effects of androgen deprivation. Data from animal studies showed that a degenerated prostate can always fully recover to its preprogrammed size regardless of the dosage and schedule of androgen administration, suggesting that the remaining basal cells may determine the growth potential of the prostate gland (28). Therefore, several investigators have postulated that basal cells represent the stem cell population (28-30). For example, using cell type-specific cytokeratin antibodies, Verhagen et al. (29) showed that luminal and basal cells share a common cell lineage in a rat prostate regeneration model. Recently, Bonkhoff et al. demonstrated using immunostaining with antibodies against proliferation-associated antigens (Kl-67, PCNA, MIB 1), that approximately 70% of the proliferative activity is confined to basal cells in both normal and hyperplastic conditions (20). Therefore, it is believed that the presence of putative homeostatic signals in basal (or stem) cells may determine the growth capacity of the prostate gland, and it is conceivable that alteration of homeostatic signals in basal cells may lead to the onset of neoplastic or malignant processes.

In this study, we demonstrated that C-CAM was associated with basal cells from the normal prostate gland. In the rat ventral prostate, we showed that C-CAM levels can be up-regulated by androgen deprivation (i.e., castration) and that the elevated levels of C-CAM remained constant in the basal epithelium throughout the entire castration period (12). The pattern of C-CAM expression contrasts with that of other androgen-repressed genes, e.g., TRPM-2, which show a transient expression after castration and are associated with apoptotic processes of the prostatic luminal epithelium (31). During the regeneration process, steady-state levels of C-CAM were detected in the luminal epithelium, suggesting that C-CAM may act as a homeostatic signal during prostate regeneration by redistributing C-CAM protein from basal cells into their progeny cells. Interestingly, Jones and Watt (32) observed that the epidermal basal (stem) cells can be distinguished from the amplifying cells and terminally differentiated epidermal cells by differences in integrin expression. Taken together, these data suggest a potential association of cell adhesion molecules with basal cell function.

In this study, overall decreases in C-CAM levels were detected in the basal cell layer from BPH glands in the transition zone and in high-grade PIN from the peripheral zone. C-CAM staining was consistently absent in all carcinomas, including tumors of transition and peripheral zone origin. The finding of decreased C-CAM expression in hyperplasia (BPH) and premalignant glands (high-grade PIN) raised the possibility that decreased C-CAM expression may be an early signal for the onset of hyperplasia (BPH) or premalignancy (PIN). The discontinuous pattern of C-CAM staining observed in 42% of the cases of high-grade PIN in our study correlates well with the established incidence of basal cell disruption observed in PIN using basal cell-specific keratin antibody (24). In their study, Bostwick and Brower (24) reported basal cell disruption in 15 and 56% of PIN2 and PIN3 lesions, respectively. Their finding in PIN3 of small glandular units protruding through areas of basal cell disruption...
(interpreted as early invasive carcinoma) also suggests that alterations in basal cells may be associated with the onset of carcinoma. In general, it is believed that two distinct signals in stem cells are crucial for maintaining homeostasis of any given tissue, i.e., proliferative signals (self-renewal) and differentiative signals (growth suppression) (28, 32). On the basis of these observations, we believe that C-CAM in basal cells may represent the signal for growth suppression. The alteration in C-CAM expression with coexisting unique zone-specific promoting factors may lead to an uncontrolled proliferative state (e.g., BPH) in the transition zone or a preneoplastic lesion (e.g., PIN) in the peripheral zone.

Acknowledgments

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References

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