

Reduced Expression of Dystroglycan in Breast and Prostate Cancer

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Cellular interactions with the extracellular matrix are an important factor in the development and progression of many types of cancer. Dystroglycan is a cell surface receptor for several extracellular matrix proteins and plays a central role in the formation of basement membranes in tissues. Because abnormalities in the structure and function of basement membranes are hallmarks of metastatic disease, we examined the status of dystroglycan expression in prostate and breast tumors. In 15 cases of surgically resected prostate cancer, we noted reduced expression of dystroglycan as judged by intensity of immunohistochemical staining. This reduction was most pronounced in high-grade disease. We found similar results in 6 cases

Dystroglycan (DG) is an integral membrane protein that links the extracellular matrix and the cytoskeleton.¹ Outside of the cell, DG binds to members of the laminin family² and the proteoglycans agrin^{3,4} and perlecan.⁵ Although first discovered in skeletal muscle as a component of the dystrophin-glycoprotein complex, it is also expressed in many nonmuscle tissues.² An underlying theme in the expression pattern of DG is that it is expressed in cell types directly adjacent to basement membranes, eg, muscle, peripheral nerve, and epithelia.⁶ Analysis of DG function has pointed to a critical role for this molecule in the formation of basement membranes. DG-null mouse embryos have structural and functional defects in the Reichert membrane, one of the first basement membranes to form during rodent embryogenesis.⁷ Moreover, DG is required for basement membrane formation in embryoid bodies derived from mouse embryonic stem cells and for the cell surface organization of laminin, an integral basement membrane protein.⁸ Taken together, available data indicate that DG binds to and organizes laminin and other matrix molecules in a manner that is important for basement membrane formation in tissues.

These findings are particularly interesting in light

of mammary ductal adenocarcinoma, suggesting that reduced expression of dystroglycan may be a conserved feature of epithelial neoplasia. These data suggest that reduced expression of dystroglycan in prostate and breast cancers may lead to abnormal cell-extracellular matrix interactions and thus contribute to progression to metastatic disease. *HUM PATHOL* 32:791-795. Copyright © 2001 by W.B. Saunders Company

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Abbreviation: DG, dystroglycan.

of the long-standing observation that in many tumors, particularly adenocarcinomas, perturbations of the basement membrane separating the epithelial and stromal compartments are common. These include thin or discontinuous basement membranes as detected by histochemistry, electron microscopy, and immunostaining for basement membrane proteins such as laminin or type IV collagen.⁹⁻¹² Moreover, some transformed cells have altered relationships with the extracellular matrix, showing decreased association of fibronectin and laminin with their surfaces compared with their normal counterparts.^{13,14}

Together, these findings contribute to the hypothesis that intact, normal basement membranes act as a physical and/or biochemical barrier to the invasion and ultimately metastasis of malignant cells. Mechanisms underlying the architectural and chemical changes in basement membranes associated with tumors are undoubtedly complex. Current research in this area explores the roles of matrix-degrading enzymes and changes in expression of matrix proteins and their receptors.¹⁵ Therefore, in this study we have begun to examine the status of DG expression in tumors of epithelial origin. We show that levels of DG protein are reduced in adenocarcinomas of the breast and prostate.

MATERIALS AND METHODS

Immunostaining

Surgically resected prostate (15) and breast (6) tumor specimens were collected at the University of Iowa Hospitals and Clinics in accordance with institutional guidelines. All studies were performed with fresh-frozen specimens. Serial sections of 7 μ m were cut and affixed to glass slides. Specimens were blocked for 1 hour in phosphate-buffered saline, pH 7.4, and 1% bovine serum albumin (Sigma, St Louis, MO). Antibodies specific for β -DG,⁷ utrophin,¹⁶ and multiple isoforms of laminin¹⁷ were diluted in blocking buffer and specimens were incubated with antibody solutions overnight. After washing with phosphate-buffered saline, primary anti-

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bodies were detected with appropriate fluorescently labeled secondary antibodies (Jackson ImmunoResearch, West Grove, PA) diluted in blocking buffer. After mounting of coverslips, specimens were analyzed by epifluorescence and/or confocal microscopy. Images were collected with an MRC 1024 laser scanning confocal microscope (BioRad, Hercules, CA). Instrument settings were held constant between benign and malignant tissues.

RESULTS

Given the prominent expression of DG in epithelial tissues, we focused our attention on two common types of tumors of epithelial origin: breast and prostate cancer. DG protein is expressed in normal human mammary epithelial cells in both ductal (Fig 1A) and lobular (data not shown) epithelium. This is consistent with previous findings on DG expression in the mouse mammary epithelium.⁶ Although DG immunoreactivity was detectable in all mammary epithelial cells, there appeared to be a somewhat stronger signal in the basal myoepithelial cells than in the apically placed luminal epithelial cells (arrows in Fig 1C). In addition to epithelial expression, DG was detectable in the fibrous stroma. We also examined the expression of an extracellular binding partner of DG, laminin, and an intracellular binding partner of DG, utrophin. Utrophin colocalized with DG throughout the ductal epithelium and in the stroma (Fig 1E). In contrast, laminin expression was restricted to the basement membrane of the epithelium and the basement membranes of blood vessels coursing through the tissue (Fig 1G). In 6 cases of ductal adenocarcinoma, we detected DG at a greatly reduced intensity in the cancer cells compared with the normal epithelium (Fig 1B, D). However, a similar level of DG signal was apparent in the stromal cells admixed with the cancer. We can rule out artifactual loss of antigen from the sample because normal epithelium present on the same sections showing regions of ductal carcinoma exhibited normal DG staining (data not shown). Utrophin showed a similar pattern as DG—decreased staining in regions of adenocarcinoma with signal persisting in the stroma (Fig 1F). Consistent with previous reports, laminin staining was virtually absent from regions of adenocarcinoma but remained detectable in blood vessel basement membranes (Fig 1H).

In benign prostatic epithelium (Fig 2A), DG protein is conspicuously detectable in the epithelium, both in basal and secretory cells, as well as in the stroma (Fig 2C). Utrophin expression is most prominent in the epithelial compartment of the prostate (Fig 2E) but could also be detected in some stromal cells (data not shown). Laminin expression was detected in the epithelial basement membrane and in association with smooth muscle elements of the stroma (Fig 2G). In 15 cases of prostate adenocarcinoma, DG was undetectable in the small, irregular pseudoglandular elements that typify mid- to high-grade disease (Fig 2B, D). However, DG expression was still detectable in the stromal elements. Likewise, utrophin expression was undetectable in the cancer but still seen in the stroma (Fig 2F).

Unlike breast cancer, and as previously described, laminin staining still outlines the small cancerous glands (Fig 2H).¹⁸ Our results show that reduced expression of DG and of its intracellular binding partner utrophin is a consistent feature of breast and prostate adenocarcinomas.

DISCUSSION

Defects in extracellular matrix organization have long been considered a hallmark of a transformed cellular phenotype and are also manifested in abnormal basement membrane structures associated with tumors. Because the extracellular matrix is involved in numerous aspects of normal tissue development, homeostasis, and physiology, these defects may promote tumor progression and metastasis rather than merely being sequelae of neoplastic disease. Underlying the extracellular matrix anomalies in cancer are imbalances in matrix-degrading enzymes and their inhibitors and changes in expression of matrix proteins and their cell surface receptors.¹⁵ Recent data indicate an important role for DG, a cell surface receptor for laminins and other extracellular matrix proteins, in the organization of laminin on the cell surface and the formation of basement membranes in early development.¹ Here we show that dystroglycan expression is significantly reduced in high-grade breast and prostate tumors, suggesting that this event might contribute to extracellular matrix disorganization in, and progression of, those diseases.

The uniformity with which DG exhibits reduced expression in the cases we examined is surprising, given the molecular heterogeneity seen in cancer. Although we have examined only a small number of cases to date, our findings indicate that loss of DG protein expression may be a conserved feature of breast and prostate cancer. This suggests that reduction of DG expression may not be caused by a primary lesion in the DG gene. Loss or mutation of a functional DG gene is important to rule out because it maps to 3p21, a chromosomal region thought to contain at least 1 tumor-suppressor gene involved in lung and other cancers.¹⁹ Alternatively, loss of DG expression may be caused by promoter methylation or proteolytic degradation. Toward the latter, an apparent breakdown product of β -DG is consistently detected in tumor cell lines but is not a common feature of DG expression in normal tissues.²⁰ More studies are needed to determine at what stage during the neoplastic process DG expression decreases and whether this event has any diagnostic or prognostic significance. It will be particularly interesting to examine DG expression in metastatic disease.

If this linkage between reduced expression of DG and progression of breast and prostate cancer can be further substantiated, it will be important to understand how a loss of DG function contributes to disease progression. Based on our current understanding of DG function, it can be speculated that DG anchors epithelial cells to basement membranes and/or serves

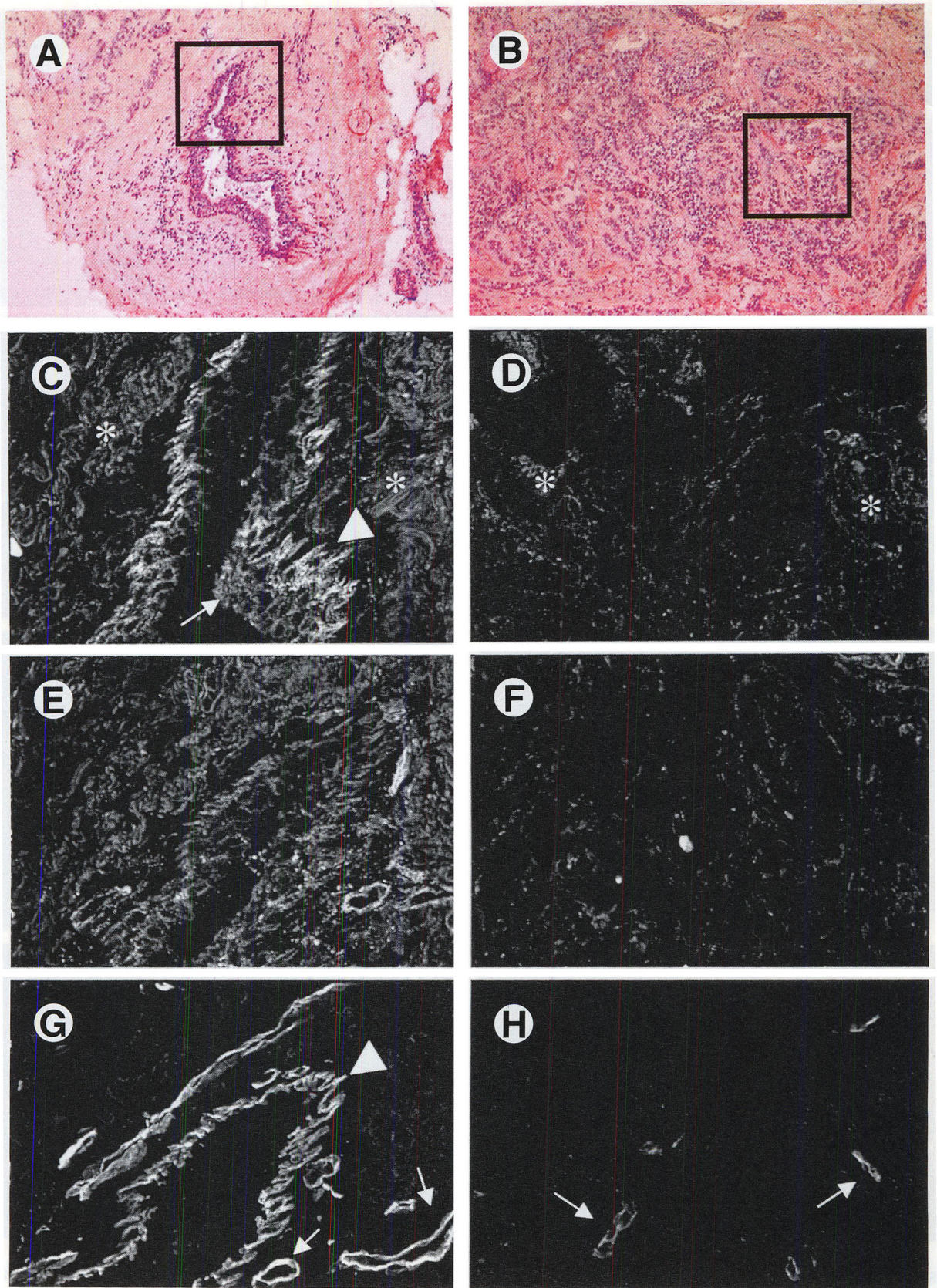


FIGURE 1. Immunofluorescence analysis of DG, utrophin, and laminin in normal and malignant mammary gland. (A) Normal mammary duct and (B) invasive ductal carcinoma (bright-field hematoxylin and eosin; original magnification $\times 10$). (C, E, G) Serial sections of A; the area shown is indicated by the black box in A (immunofluorescence; original magnification $\times 40$). (D, F, H) Serial sections of B; the area shown is indicated by the black box in B (immunofluorescence; original magnification $\times 40$). (C) DG expression is detectable in normal ductal epithelium in both apical epithelial cells (arrow) and myoepithelial cells (arrowhead) and in the stromal compartment (areas surrounding asterisks). (D) In ductal adenocarcinoma, some DG staining persists in the stroma (asterisks). (E, F) Utrophin staining, like DG, is also detectable in the epithelial and compartments and shows decreased expression in adenocarcinoma. (G) Laminin is detectable in the basement membrane of the normal duct (arrowhead) and in vascular basement membranes (arrows). (H) In ductal adenocarcinoma, laminin is undetectable, but blood vessels coursing through the specimen still show staining in their basement membranes (arrows).

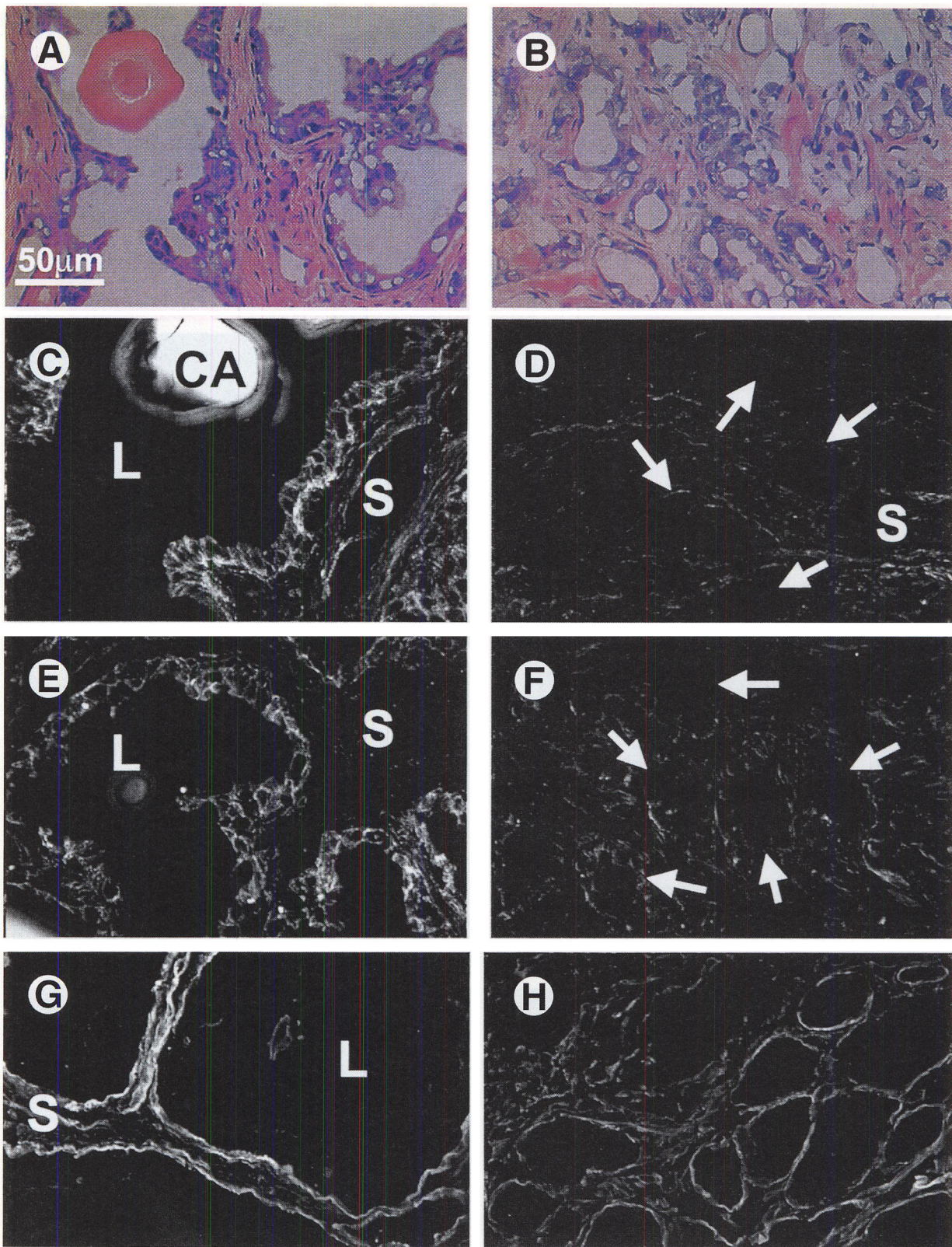


FIGURE 2. Immunofluorescence analysis of DG, utrophin, and laminin in benign and malignant prostatic epithelium. (A) Benign prostatic epithelium and (B) prostate cancer (Gleason grade 3) (bright-field hematoxylin and eosin; original magnification $\times 40$). (C, E, G) Serial sections of A showing a similar, but not the same, field; L, lumen; S, stromal compartments of the gland for reference (immunofluorescence, original magnification $\times 40$). (D, F, H) Serial sections of B showing a similar, but not the same, field; L, lumen; S, stromal compartments of the gland for reference (immunofluorescence; original magnification $\times 40$). (C) DG expression is detectable in benign prostatic epithelium and appears to be in both secretory and basal cells. DG is also detected in the stromal compartment most prominently with the smooth muscle. The asterisk marks the position of a corpus amylacium. (D) In prostate adenocarcinoma, some DG staining persists in the stroma (asterisks), but little DG is detectable in the regions of adenocarcinoma. Arrows in D and F mark the position of the small acinar structures characteristic of prostate cancer. (E, F) Utrophin staining, like DG, is also detectable in the epithelial and compartments and shows decreased expression in adenocarcinoma. (G) Laminin is detectable in the basement membrane of the benign glands and in association with smooth muscle. (H) In prostate adenocarcinoma, unlike ductal adenocarcinoma of the breast, laminin is detectable and the cancerous lesion is associated with the small, irregular acinar structures.

to structurally organize basement membranes. Derangement in either function may be a contributing factor in the progression of cancer. Moreover, it is important to explore how loss of DG function may cooperate with mechanisms relating to abnormalities in expression of other extracellular matrix receptors, such as integrins.²¹

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