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Role of the Extracellular Matrix in Prostate Carcinogenesis

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Abstract This review summarizes the current state of knowledge regarding the proteins composing the extracellular matrix in the human prostate. The normal expression as well as the changes which occur in PIN and carcinoma are described for the lamins, collagens, and glycosaminoglycans. J. Cell. Biochem. 91: 36–40, 2004. © 2003 Wiley-Liss, Inc.

Key words: extracellular matrix; prostate; laminins; collagen IV

The extracellular matrix (ECM) in the prostate is composed of a diverse and complex array of proteins distributed in the interstitium as well as forming the basal laminae (BL) surrounding the various components of the prostate. Although little is known of the precise role of many of these proteins in the prostate, we know from experimental systems that they function in such vital roles as development, cell adhesion, cell migration, cell differentiation, and cell survival. They function not only as structural barriers which separate various cell populations, but also act as important ligands for transmembrane receptors and therefore provide important outside-in signals for stromal–epithelial interactions [Bosman and Stamenkovic, 2003]. Evidence has recently emerged that they not only sequester important growth factors but also, upon cleavage, may produce biologically active fragments [Shenk et al., 2003]. In this article, we will review what is currently understood about the prostate ECM and the changes known to occur in prostate carcinoma progression.

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ECM COMPONENTS IN NORMAL PROSTATE

The human prostate is composed of three major subdivisions: the transitional zone surrounding the proximal urethra; the central zone; and the peripheral zone [McNeal, 1980]. These zones are remarkably different in their disease susceptibility in that benign prostatic hyperplasia (BPH) affects primarily the transitional zone whereas prostate cancer occurs primarily in the peripheral zone. The development of the various zones is different and results in a ductal drainage pattern, which is different. Subtle differences in the glandular architecture have been described for the various zones, however, thus far there has not been a study examining whether there are differences in the ECM of the various zones.

COMPONENTS OF NORMAL BL

BL are found surrounding the intraprostatic urethra, ducts glands as well as the stromal smooth muscle, nerves, and vessels. The normal glands are surrounded by a thin, 80 nm basal lamina. The major components of the prostate epithelial BL are similar to other epithelial BLs and include: Type IV collagen, entactin, laminins, and proteoglycans, especially hyaluronic acid (HA). In addition, the normal glandular prostate BL contains tenascin, Type VII collagen, vitronectin, and fibronectin [Knox et al., 1994].

The laminins constitute a superfamily of glycoproteins [Colognato and Yurchenco, 2000]. Each specific laminin isoform is composed of a unique combination of three separate pro-

teins (α, β, and γ chains). The three chains form a coiled-coil helix with the three projecting N-terminal globular domains forming a cruciform structure [Salo et al., 1999]. Thus far, 11 genetically distinct laminin chains have been described, α1–α5, β1–β3, and γ1–γ3 [Burgeson et al., 1994; Patton et al., 1997; Koch et al., 1999]. Thirteen laminin isoforms have been convincingly demonstrated, although up to 45 forms are theoretically possible [Patton et al., 1997; Ferletta and Ekblom, 1999; Koch et al., 1999; Libby et al., 2000].

We have discovered that the laminin chains α1, α2, α3, α5, β1, β2, γ1, and γ2 are all expressed in normal prostate glands [Nagle et al., 1994; Brar et al., 2003]. The α1 chain (laminins 1/3) is prominent in fetal and newborn prostate but in the adult is replaced by α3 (laminins 5, 6, 7) and α5 (laminins 10/11) [Brar et al., 2003]. Laminin 5 (α3β3γ2) forms anchoring filaments which span the basal lamina connecting the α6β4 integrin with its C-terminal end and binding to collagen VII, forming the anchoring fibrils with its N-terminal end [Rosselle et al., 1997]. Thus, laminin 5 functions as a major component of the hemisdesmosome anchoring the prostatic basal cells to basal lamina. Laminin 5 forms filamentous structures by lateral associations with itself or with laminins 6 and 7, but it does not bind entactin and therefore does not directly form a complex with collagen IV. α1 and α5 can interact with collagen IV through entactin and are therefore believed to be major components of the felt-like array of molecules forming the lamina densa. The α5 chain, in addition to being a major component of prostatic epithelial BL, is also expressed surrounding smooth muscle cells and vascular endothelium in the interstitium [Brar et al., 2003]. The α4 chain (laminins 8 and 9) is expressed by prostate stromal vessels and smooth muscle but is not seen in epithelial basal lamina. Entactin is, as expected, also expressed in normal glandular basal lamina.

Collagen IV is a trimer formed of three chains. Six specific subchains (α1–α6) can combine to form the collagen IV trimer in human BL [Sundaramoorthy et al., 2002]. An analysis of the prostate reveals that normal glands express extensive amounts of α1, α2, α5, and α6 with lesser amounts of α3. The α4 chain is not expressed.

HA is a glycosaminoglycan made up of repeated disaccharide units, D-glucuronic acid and *N*-acetyl-D-glucosamine [Delpech et al., 1997]. It plays a major role in the hydration of tissue matrix and maintains osmotic balance [Tammi et al., 2002]. Through interaction with its receptors (CD44 and RHAMM) it controls cell adhesion, migration, and cell proliferation [Turley et al., 2002]. HA is degraded by hyaluronidase into small angiogenic fragments of 3–25 disaccharide units [West et al., 1985]. HA is not seen in normal prostate epithelium but is seen weakly expressed in the normal prostate stroma both intra- and extra-cellularly [Lipponen et al., 2001].

CHANGES IN ECM COMPONENTS WITH PROSTATE CARCINOMA PROGRESSION

It is now generally accepted that prostatic intraepithelial neoplasia (PIN) is a precursor to invasive cancer [McNeal and Bostwick, 1986]. One of the hallmarks of PIN is the focal attenuation or loss of the basal cell layer, which is completely lost in even low-grade invasive carcinoma. In the PIN lesions, the basal lamina underlying the retained basal cells contains all the elements of the normal basal lamina [Nagle et al., 1995], however, in areas where the basal cells are lost there is also lost expression of laminin 5 [Davis et al., 2001]. Recent studies have shown that in these areas of basal cell loss, there is also loss of the α5 and α6 subchains of collagen IV (unpublished data).

In invasive prostate carcinoma several elements of the normal hemidesmosome are not polarized to the basal lamina. The β4 integrin, BP180, collagen VII anchoring fibers, and the laminin 5 anchoring filaments are all lost [Nagle et al., 1995]. The α6 integrin loses its polarity and is seen expressed throughout the cytoplasmic membrane, probably associated with its default partner, the β1 integrin.

The loss of laminin 5 in prostate carcinoma deserves special comment since, in a number of other human neoplasms, laminin 5 chains are actually expressed at the invading edge and are therefore thought to be important in promotion of neoplastic cell invasiveness similar to its role in wound healing [Hida et al., 1994; Pyke et al., 1995; Davis et al., 2001]. We have demonstrated that the exposure of PO145 prostate cells to laminin 5 cause extensive changes in gene expression [Calaluce, 2001]. We have investigated the loss of laminin 5 in prostate carcinoma and demonstrated that the α3 chain is expressed,

but neither the γ2 nor the β3 chains were expressed [Hao et al., 2001]. Surprisingly, when the mRNAs for the three chains were investigated by in situ hybridization, all three were expressed not only in normal basal cells but also in the cells forming the glandular structure in prostate carcinoma. Laser capture microdissected specimens were used to confirm the presence of all three mRNAs by reverse Northern analysis [Hao et al., 2001]. An attempt to identify the reason why these messages were not translated using LNCap cells revealed two forms of the β3 message with dissimilar 5′ untranslated regions. These mRNA isoforms arise by alternate start sites on the *β3* gene exon 1. Premature stop codons and missense mutations were not found. An analysis of a number of cell lines revealed differential expression patterns for these two forms of the mRNA and revealed that only cells expressing the β3A form of the message translated the β3 protein [Hao et al., 2002]. Microdissected samples of human prostate carcinoma revealed variable presence of both mRNA forms, although none of these tumors translated the β3 protein. Stable transfection of the β3A form of the mRNA but not the β3B form into LNCaP cells restored the production of β3 protein (Calaluce, unpublished data). Both forms of the mRNA are translated in the rabbit reticulocyte system. Apparently, prostate cancer cells can transcribe both forms of the message but lack elements necessary for translation.

The basal lamina surrounding prostatic carcinoma is rich in laminin 10/11 which correlates with the finding that the main integrins persisting in carcinoma are α3β1 and α6β1, the major integrin receptors for laminin 10 [Schmelz et al., 2002]. This basal lamina forms the final barrier to stromal invasion. The stromal vasculature as well as the basal lamina surrounding the muscle cells in the stroma are also rich in laminin 10 as is the bone marrow stroma. This suggests that carcinoma cells are resting on a laminin 10 rich basal lamina and are already adapted to exist in the stromal environment.

Early ultrastructural studies had indicated that the basal lamina surrounding prostate carcinoma was focally disrupted and the degree of disruption was related to increasing tumor grade [Fuchs et al., 1980].

Recent studies carried out in collaboration with Dr. Tim Bowden's laboratory have revealed that prostate carcinoma has upregulated expression of MT1-MMP [Udayakumar et al.,

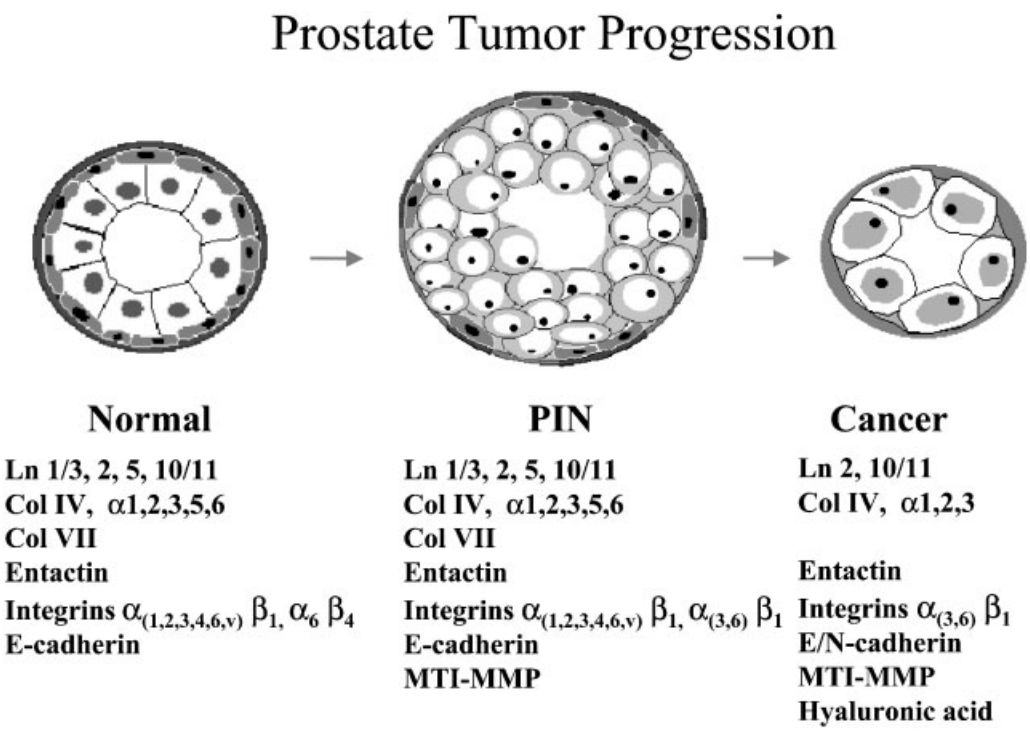

Fig. 1. Prostate tumor progression [adapted from Nagle et al., 1994].

2003]. In vitro studies indicate that MT1-MMP selectively cleaves the α5 chain of laminin 10/11 and increased cell migration and cell invasiveness (unpublished data).

Additional recent observations indicate that the α5 and α6 subchains of collagen IV are also lost in carcinoma (unpublished observations). Whether this is a transcriptional or a translational defect has not been determined but may be related to the fact that the α1β1 integrin, the collagen IV receptor, is also lost [Schmelz et al., 2002].

HA has been shown to manifest increased expression both in tumor cells and in peritumoral stroma in prostate cancer [Lipponen et al., 2001; Lokeshwar et al., 2001; Posey et al., 2003]. Lokeshwar et al. [2001] reported that primary prostate carcinoma epithelial cells and stromal fibroblasts secreted 3–8 fold more HA than normal or BPH cultures. These authors also reported immunohistochemical analysis of prostate tissue showing increased HA as well as hyaluronidase (HYAL1) in prostate carcinoma with respect to normal and BPH tissue. Lipponen et al. [2001], investigated HA and its receptor CD44. The higher level of HA in peri-tumoral tissue was related to metastasis, higher T-stage, high Gleason score, perineural infiltration, and high mitotic activity of the tumor (for all $P < 0.001$). There was an inverse relation of the HA receptor (CD44) to high T-stage, high Gleason score, and high mitotic activity (for all $P < 0.001$).

CONCLUSIONS

Our knowledge of even the normal components of the ECM in human prostate tissue is far from complete. In this review, we have summarized some of the complexities of the prostate ECM and have given an indication that these are dynamic features of the prostate tissue, which undergo change during prostate carcinogenesis (see Fig. 1 for summary). Given the emerging realization of the dynamic signaling which goes on between the stroma and the epithelia [Cunya et al., 2002; Shian-Yang and Chung, 2002; DeWever and Marceel, 2003], it is clear that a more complete understanding of the ECM will be necessary in order to devise preventive and therapeutic intervention strategies aimed at combating prostate hyperplasia and neoplasia.

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