Journal of Cellular Biochemistry, Supplement 16H:26-29 (1992)

New Relationships Between Prostatic Intraepithelial Neoplasia and Prostatic Carcinoma

Ray B. Nagle, M.D.[1], Michel Petein, M.D.[2], Michael Brawer, M.D.[3], G. Tim Bowden, Ph.D.[4], and Anne E. Cress, Ph.D.[4]

[1] Department of Pathology, University of Arizona Health Sciences Center, Tucson, Arizona 85724
[2] Department of Pathology, Institute J. Bordet, Brussels, Belgium
[3] Veterans Administration Medical Center, Seattle, Washington 98108
[4] Department of Radiation Oncology, University of Arizona Health Sciences Center, Tucson, Arizona 85724

Abstract Our group has been studying the progressive molecular changes in prostatic epithelium which precede the invasive phenotype. Initial studies revealed similar alterations in cytoskeletal proteins between high grade prostatic intraepithelial neoplasia (PIN) lesions and invasive carcinoma. Specifically we observed an increased expression of certain cytokeratins and decreased expression of vimentin. We also noted a change in glycosylation as detected by *Ulex europaeus* staining. Using the latter technique we were able to microdissect and isolate nuclei from areas of low and high grade PIN lesions as well as from invasive carcinoma for morphometric analysis. Similarities in nuclear size, chromatin heterogeneity, and nuclear DNA content between low and high grade PIN and invasive carcinoma in carcinomatous specimens were noted. In contrast, these parameters were significantly different in low grade PIN lesions obtained from benign prostatic transurethral resection (TURP) specimens. In addition, DNA histograms revealed similar proliferative indices between high grade PIN and invasive carcinoma, which differed significantly from low grade PIN. Parameters thought to be relative to the invasive phenotype were also examined, such as the members of the metalloproteinase family; although normal luminal cells fail to express detectable levels of these enzymes, invasive carcinoma and even low grade PIN lesions express both the 72 kDa and 92 kDa type IV collagenase. Taken together, these data indicate that the dysplastic cells of PIN lesions and carcinomas are similar in nuclear and genomic features as well as protease expression. Our current working hypothesis is that these cells are already armed with the necessary proteases to invade the basal lamina but in an inactive form. Tumor progression requires an additional event of protease activation.

Key Words: lectins, metalloproteinase, PIN, proliferative indices, prostate carcinoma, thymidine labeling, tumor progression

Atypical or dysplastic lesions of the prostatic ducts and acini were originally described by Oertel in 1925 [1]. Since that time a variety of lesions have been described using a confusingly long list of terms (see Ref. 2 for review). Kovi was the first author to separate lesions of the large acinar type from atypical lesions involving the abnormal hyperplasia of small acini [3]. The dysplastic lesions of the large acinar type have cytologic features which in their most severe form resemble carcinoma *in situ*. McNeal and Bostwick have described the histologic features of these lesions and have characterized three grades with increasing cytologic atypia [4]. In a subsequent paper, Bostwick and Brawer showed a progressive loss of basal cells with increasing grade of atypia and have proposed the term prostatic intraepithelial neoplasia (PIN) for these lesions [5].

The exact relationship of PIN lesions to invasive carcinoma remains unknown, however. PIN lesions are found in the peripheral prostate lobe [3], are multifocal [6,7], and when high grade are often found in association with invasive carcinoma [5,7]. Thymidine labelling studies have shown increased labelling of PIN when compared with simple prostatic hyperplasia [8]. A number of recent immunohistochemical studies have shown progressive loss of markers of prostatic glandular secretion in the PIN lesions, implying a loss of glandular differentiation [9–13]. We have examined the intermediate

filament expression in the normal prostate glands, PIN, invasive carcinoma, and prostate cell lines [2,14]. Using a monoclonal antibody (KA4) which reacts with cytokeratins 14, 15, 16, and 19, we were able to show that the normal acinar luminal cells, which express only cytokeratins 8 and 18, were nonreactive; in contrast, the cells of high grade PIN as well as invasive carcinoma were reactive. This appeared to be due to an increase in cytokeratin 19 expression. In addition, whereas the luminal cells normally coexpress cytokeratins and vimentin, this ability is lost in PIN and invasive cancer. These two findings indicate major changes in cytoskeletal protein expression which accompany the more obvious changes in nuclear morphology.

The lectin *Ulex europaeus* has been shown by our group as well as others to selectively stain PIN epithelial cells [2,12]. Using this method to identify the lesions we were able to microdissect out specific lesions from which nuclear suspensions were Feulgen-stained and placed on cytospin slides for image analysis with a SAMBA 200 system [15,16]. Morphometric, nuclear textural, and densitometric parameters related to DNA content were evaluated in four groups of lesions: (1) low grade 1 and 2 PIN lesions from benign TURP specimens, (2) low grade 1 and 2 PIN lesions from cancer-containing prostatectomy specimens, (3) high grade 3 PIN lesions from cancer-containing prostatectomy specimens, and (4) invasive carcinoma [17].

The analysis of the four groups revealed a relationship between low grade PIN, high grade PIN and invasive carcinoma when these lesions occurred in cancerous prostates. These were all significantly different in terms of nuclear size, mean DNA content, and nuclear texture when compared to low grade PIN lesions found in nonmalignant prostates. This finding suggests that there is a field effect which is already manifest in low grade PIN lesions in men having a predilection for invasive carcinoma. Analysis of DNA histograms revealed a further relationship. High grade PIN lesions and invasive carcinomas had relatively large S-phase fractions that were statistically different from low grade PIN lesions whether they were in benign or in malignant prostates. These data are similar to the earlier studies with thymidine labelling showing that high grade PIN lesions had higher levels of proliferation in relation to hyperplastic lesions [8]. Based on these findings, there appear to be clear differences between low grade PIN found in benign and malignant prostates. Whether or not the low grade PIN lesions in malignant prostates progress to high grade lesions and then to invasive carcinoma remains to be seen. The biochemical data showing similarities in intermediate filament expression suggest that at least the high grade lesions are similar to invasive carcinoma and are probably on the progression pathway. Studies showing a sequence of predictable genetic events are thus far not available for prostate. These concepts are summarized in Figure 1.

The major difference between grade 3 PIN lesions and invasive carcinoma is the loss of the basal cell layer and penetration of the underlying basal lamina in the latter. This process is believed to occur through a three-step process involving cellular adhesion, lysis of the basal lamina, and migration into the interstitium. We have investigated the second parameter, that is, lysis of the basal lamina, by examining the expression of the multigene family of metalloproteinases. These metalloproteinases may play a functional role in the loss of the basal cell layer and penetration of the basal lamina by the tumor cells. To date seven separate metalloproteinases described in this family are found in human tissues [18,19]. Our preliminary data using a combination of northern RNA analysis, *in situ* RNA hybridization [20], and immunohistochemistry [21], have indicated that three of these proteins, specifically the 72 kDa and 92 kDa collagenase type IV and matrilysin or PUMP-1, are observed in primary prostate carcinoma. Immunohistochemical studies indicate that both the 72 kDa and 92 kDa type IV collagenases are expressed by most carcinomas. Most interestingly, PIN lesions also constitutively express these metalloproteinases, and at least the 72 kDa type IV collagenase shows an increasing degree of expression with increasing grade of PIN. In contrast, the 72 kDa type IV collagenase shows a decreasing expression with increasing grade of carcinoma. The 92 kDa type IV collagenase does not show increasing expression in the PIN lesions, but shows increasing expression as the Gleason grade of carcinoma increases. It thus appears that the expression of these two enzymes is under differ-

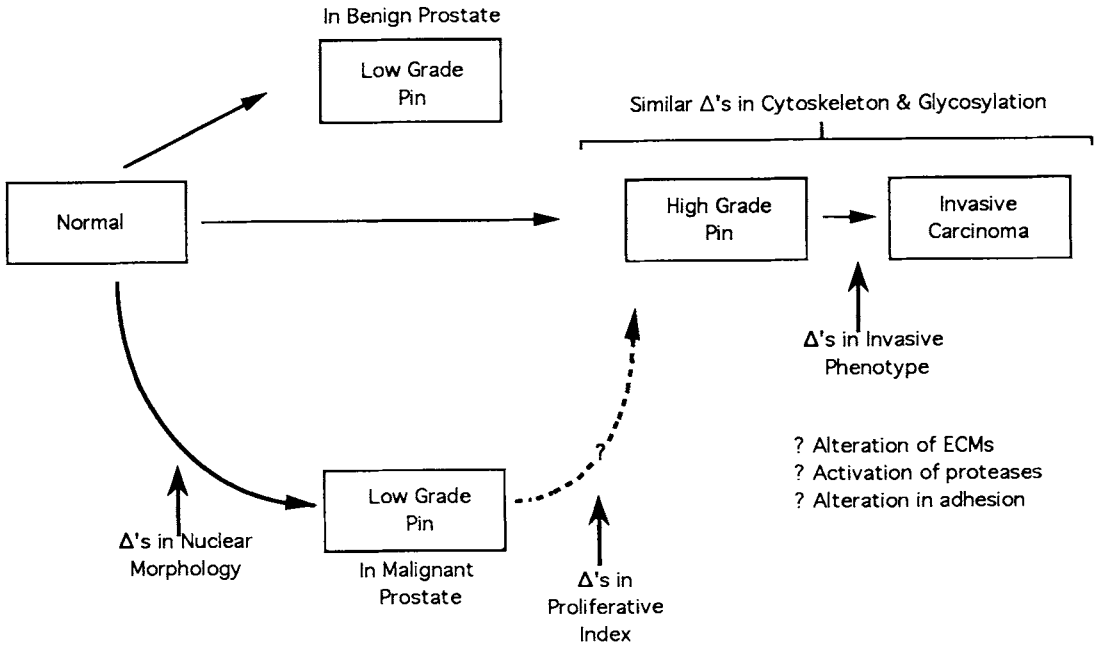

Fig. 1. Concepts of Prostate Tumor Progression

ent control but that both are constitutively turned on in the preinvasive lesion. The antibodies used in these studies could not distinguish pro-enzyme from active enzyme; therefore, the collagenase enzymes may be present either in active or pro-enzyme forms. Alternatively, they may be completely inhibited by tissue inhibitors of metalloproteinases. These inhibitors are known to be closely associated and coordinately expressed with the enzymes. It would appear that activation of these collagenases must require an additional event or events, and it is likely that the discovery of this control will be important in our understanding of the steps in the progression of preinvasive high grade PIN lesions to the invasive carcinoma.

REFERENCES

1. Oertel H: Involutionary changes in prostate and female breast in relationship to cancer development. Can Med Assoc J 16:237–241, 1925.
2. Nagle RB, Brawer MK, Kittelson J, Clark V: Phenotypic relationships of prostatic intraepithelial neoplasia to invasive prostatic carcinoma. Am J Pathol 138:119–128, 1991.
3. Kovi J, Mostofi FK, Heshmat MY, Enterline JP: Large acinar atypical hyperplasia and carcinoma of the prostate. Cancer 61:555–561, 1988.
4. McNeal JE, Bostwick DG: Intraductal dysplasia: A premalignant lesion of the prostate. Hum Pathol 17:64–71, 1986.
5. Bostwick DG, Brawer MK: Prostatic intraepithelial neoplasia and early invasion in prostatic cancer. Cancer 59:114–120, 1987.
6. Kastendieck H: Correlations between atypical primary hyperplasia and carcinoma of the prostate. Pathol Res Pract 169:366–387, 1980.
7. Troncoso P, Babaian RJ, Ro JY, Grignon DJ, von Eschenbach AC, Ayala AG: Prostatic intraepithelial neoplasia and invasive prostatic adenocarcinoma in cytoprostatectomy specimens. Urology 34:52–56, 1989.
8. Helpap B: The biologic significance of atypical hyperplasia of the prostate. Virchows Arch [A] 387:307–317, 1980.
9. Ellis DW, Lefers S, Davies JS, Ng ABP: Multiple immunoperoxidase markers in benign hyperplasia and adenocarcinoma of the prostate. Am J Clin Pathol 81:279–284, 1984.
10. McNeal JE, Leav I, Alroy J, Skutelsky E: Differential lectin staining of central and peripheral zones of the prostate and alterations in dysplasia. Am J

Clin Pathol 89:41–48, 1988.
11. McNeal JE, Alroy J, Leav I, Redwine EA, Freiha FS, Stamey TA: Immunohistochemical evidence of impaired cell differentiation in the premalignant phase of prostate carcinogenesis. Am J Clin Pathol 90:23–32, 1988.
12. Perlman EJ, Epstein JI: Blood group antigen expression in dysplasia and adenocarcinoma of the prostate. Lab Invest 60:72A, 1989.
13. Reese JH, McNeal JE, Redwine EA, Stamey TA, Freiha FS: Tissue type plasminogen activator as a marker for functional zones, within the human prostate gland. Prostate 12:47–53, 1988.
14. Nagle RB, Ahmann FR, McDaniel KM, Paquin ML, Clark VA, Cleniker A: Cytokeratin characterization of human prostatic carcinoma and its derived cell lines. Cancer Res 47:281–286, 1987.
15. Van Driel-Kulker A, Mesker W, Van der Burg M, Ploem J: Preparation of cells from paraffin-embedded tissue for cytometry and cytomorphologic evaluation. Anal Quant Cytol Histol 9:225–231, 1987.
16. Brugal G, Garbay C, Giroud F, Adhel D: A double scanning microphotometer for image analysis: Hardware, software and biomedical applications. J Histochem Cytochem 27:144–152, 1979.
17. Petein M, Michel P, Van Velthoven R, Pasteels J-L, Brawer MK, Davis JR, Nagle RB, Kiss R: Morphonuclear relationship between prostatic intraepithelial neoplasia and cancers assessed by digital cell image analysis. Am J Clin Pathol 96:628–634, 1991.
18. Matrisian LM: Metalloproteinases and their inhibitors in matrix remodeling. TIG 6:121–125, 1990.
19. Woessner JF: Matrix metalloproteinases and their inhibitors in connective tissue remodeling. FASEB J 5:2145–2154, 1991.
20. Siadat-Pajouh M, Nagle RB, Breathnach R, Finch JS, Brawer MK, Bowden GT: Expression of metalloproteinase genes in human prostate cancer. J Cancer Res Clin Oncol 117:144–150, 1991.
21. Nagle RB *et al.*: Unpublished data.