The Significance of Focal Basal Cell Layer Disruption-Induced Immuno-Cell Infiltration in Prostate Cancer Invasion

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Using multidisciplinary approaches, our studies assessed the physical status of prostate basal cell layers and the impact of basal cells on the biological presentation of associated epithelial cells. Our studies showed that focally disrupted basal cell layers had the following unique features: [1] significantly lower proliferation; [2] significantly lower p63 expression; [3] significantly higher apoptosis; [4] significantly higher leukocyte infiltration and stromal reactions. In contrast, epithelial cells overlying focal basal cell layer disruptions (FBCLD) showed [1] significantly higher proliferation; [2] significantly higher expression of tumor invasion-related genes; [3] direct physical continuity with invasive lesions. Based on these and other findings, we have proposed that prostate tumor invasion is triggered by a localized degeneration of aged or injured basal cells and resultant inflammatory reactions, and that cells overlying FBCLD represent the direct precursor of invasive lesions. Our hypothesis has been recently published in multiple peer-reviewed journals.

Parallel Synthesis: Biocatalytic Amplification; Drug Discovery; Chemotherapeutics; Lead Lead Optimization
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**Introduction**

The prostate luminal cells, which are the histological origin of a vast majority of prostate malignancies, are physically separated from the stroma by basal cells and the basement membrane (BM). Basal cells are joined by intercellular junctions and adhesion molecules, forming a continuous sheet that encircles ducts and acini (Fig 1) (1-2). The BM is composed of type IV collagen, laminins, and other molecules, forming a continuous lining surrounding and attaching to the basal cell layer (3-4). The epithelium is devoid of blood vessels and lymphatic ducts, and is therefore totally dependent upon the stroma for its normal functions and even survival. Due to this structural relationship, the disruption of both the basal cell layer and BM is pre-requisite for prostate tumor invasion or metastasis.

![Fig 1](image_url)

**Invasive lesion**

EP cells
Basal cells
BM
ST

**Body**

Promoted by the reports that: [1] basal cells are the source of several tumor suppressors, including p63 and maspin, [2] the absence of basal cell layer is the most distinct feature of invasive lesions, and [3] chronic inflammation promotes prostate cancer (14-18), our recent studies have attempted to identify the early alterations of basal cell layers and their potential impact on prostate tumor invasion. Using a double immunostaining method with antibodies to cytokeratin (CK) 34ßE12 (a basal cell phenotypic marker), our initial study assessed the physical integrity of basal call layers in paraffin-embedded tumor (n=50) prostate tissues with co-existing pre-invasive and invasive components (19). Of 2,047 ducts and acini examined, 201 were found to contain focal disruptions (the absence of basal cells resulting in a gap larger than the combined...
size of at least 3 basal cells) in surrounding basal cell layers. The frequency of focal disruptions (FBCLD) varied substantially among cases (Table 1).

Table 1. Frequencies of focal basal cell layer disruptions among different cases

<table>
<thead>
<tr>
<th>Case number</th>
<th>No disruptions</th>
<th>1-10% disruptions</th>
<th>&gt; 30% disruptions</th>
<th>p</th>
</tr>
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<tr>
<td>50</td>
<td>22 (44%)</td>
<td>11 (22%)</td>
<td>17 (34%)</td>
<td>&lt; 0.01</td>
</tr>
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</table>

Compared to their non-disrupted counterparts, focally disrupted basal cell layers showed the following unique features: [1] significantly lower proliferation; [2] significantly lower p63 expression; [3] significantly higher apoptosis; [4] significantly higher leukocyte infiltration and stromal reactions.

Compared to their counterparts distant from focal disruptions or overlying the non-disrupted basal cell layers, epithelial cells overlying FBCLD showed the following unique features: [1] significantly higher proliferation; [2] significantly higher gene expression (Fig 2); [3] physical continuity with adjacent invasive lesions.

![Fig 2. Comparison of gene expression between cells overlying focal BCLD and adjacent cells within the same duct. Cells were microdissected and subjected to RNA extraction, amplification, and gene expression profiling using our published protocols. Circles identify microdissected cells overlying focal BCLD & differentially expressed genes. Squares identify microdissected adjacent cells.

Among a total of 600 different genes assessed using the Pathway-focused oligo DNA micro-arrays, 23 genes were significantly and differently (at least 5-fold difference) expressed between cells overlying FBCLD and the
adjacent cells within the same duct. Of these, genes-specific for extracellular matrix proteinases, interleukins and their corresponding receptors were significantly lower in cells overlying FBCLD, which provides additional evidence that the proteolytic enzyme theory might not reflect the intrinsic mechanism of tumor invasion. In contrast, cells overlying FBCLD had significantly higher expression levels in several gene groups, including those for cell proliferation, anti-apoptosis, and stem cells (20; Fig 2). All these elevated genes have been shown to directly promote tumor progression and invasion.

Together, these findings suggest that focal basal cell layer disruptions could substantially impact the molecular profile and biological presentations of the overlying epithelial cells. Based on these and other findings, we have proposed that prostate tumor invasion is triggered by a localized degeneration of aged or injured basal cells and the resultant auto-immunoreactions. Our hypothesized steps for prostate tumor invasion include the following: [1] due to inherited or environmental factors, some patients contained cell cycle control- and renewal-related defects in the basal cell population that cause elevated basal cell degenerations; [2] the degradation products of degenerated basal cells or diffusible molecules of the overlying epithelial cells attract leukocyte infiltration; [3] leukocytes discharge their digestive enzymes upon the direct physical contact, resulting in a focal disruption in the basal cell layer, which leads to several focal alterations:

a. A localized loss of tumor suppressors and paracrine inhibitory function, which confers tumor cell growth advantages to escape the programmed cell death (21-25).

b. A localized increasing of permeability for nutrients and growth factors, and altered oxygen level, which selectively favors the proliferation of progenitor or stem cells (26-28).

c. A localized increasing of leukocyte infiltration, which directly export growth factors to tumor cells through direct physical contact (29-33).

d. The direct tumor-stromal cell contact, which augments the expression of stromal MMP or represses the expression of E-cadherin and other epithelial cell specific markers, which facilitates epithelial-mesenchymal transition (34-36).

e. The direct exposure of the overlying epithelial cells to the stromal tissue fluid, which might dilute the adhesion molecules on the surface of the epithelial cells.

These alterations could individually or collectively lead to increasing proliferation and motility in overlying epithelial cells that lead to the stromal invasion of the cells overlying FBCLD.

Our hypothesis differs from the traditional theories in six main aspects: [1] the triggering factor for the initiation of tumor invasion, [2] the stage of tumor invasion, [3] the cellular origin of invasive lesions, [4] the significance of immunoreactive cells, [5] the significance of stromal cells, and [6] potential approaches for early detection, treatment, and prevention of tumor invasion. Our hypothesis represents a novel in vivo model as to the cellular mechanism leading to prostate tumor invasion. If confirmed, it could have an immediate impact on patient care through improved pathologic evaluation of prostate tumor biopsies. More broadly, the results of our study may lead to the development of more effective and specific approaches for prostate cancer detection, treatment, and prevention.

**Key research accomplishments**

1. All the laboratory procedures for all Tasks listed had been completed.
2. A total of 8 manuscripts and abstracts have been published, accepted, or submitted, and two are in preparation.
3. A total of 23 significantly and differentially (at least 5-fold) expressed genes have been identified between cells overlying focally disrupted basal cell layers and adjacent cells.
4. A novel hypothesis of prostate tumor progression and invasion has been introduced.

**Reportable outcomes**

Three manuscripts and 5 abstracts have been published or accepted for publication, and two manuscripts
are in preparation (please see below)

**a. Manuscripts:**

**b. Abstracts:**
5. Schwartz AM, Man YG, Rezaei MK, Berg PE. BP1, a homeoprotein, is significantly expressed in prostate adenocarcinoma and concordant with prostatic intraepithelial neoplasia. Proc Am Assoc Cancer Res 48, in press.

**Conclusions**
The results of our current study are in total agreement with our previous hypothesis, further suggests that prostate tumor invasion is triggered by a localized degeneration of aged or injured basal cells and the resultant auto-immunoreactions.

**References**

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Man, Yan-Gao CONTR

From: Wood, Kimberly M.
Sent: Monday, March 26, 2007 3:05 PM
To: Man, Yan-Gao CONTR
Subject: RE:

Hi Dr. Man:
The grant ID # for this grant is W81XWH-0610382 (ARP #7031).
ARP would be very happy to receive a no extension for another 8 months to a 1 year with USAMRAA's permission.

If you need anything else from me, I will be happy to help -Kim

******************************************************************************
Kim Wood
Contract and Human Resource Manager
American Registry of Pathology
1413 Research Blvd
Rockville, MD 20850
phone: 301.319.0084
fax: 301.319.0620

-----Original Message-----
From: Man, Yan-Gao CONTR
Sent: Monday, March 26, 2007 4:01 PM
To: Wood, Kimberly M.
Subject:

Kim,
Although all the experimental procedures have been accomplished and the main findings have been published, I have about $55,000 left in my account. I would like to keep this money for additional 8-12 months for three main reasons:
1. There are 4 additional manuscripts in press or in preparation, which contain a total of over 50 plates of color figures. The price for publishing a color figure plate is about $500.
2. For our submitted manuscripts, we may have to carry out additional experimental procedures demanded by the reviewers.
3. The expenses for the coming “DOD prostate cancer research program meeting” have not been paid.

Yan-gao