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Heterogeneous Expression of Invasive and Metastatic Properties in a Prostate Tumor Model

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Cellular heterogeneity of neoplasia is well demonstrated in the Dunning R-3327 rat prostate adenocarcinoma. In this study, we measured the differential expression of invasive and metastatic properties of this prostate model by cloning from a heterogeneous parental cell line. Four cell clones were derived and characterized by morphological studies, E-cadherin expression, and invasive and metastatic potential. Three of the clones (clones 5'A, 5'C, and 5'D) demonstrated a fibroblastic morphology and were anchored to the substrate by loose microvillous processes. The fourth clone (clone 5'B) grew in tight clusters and displayed many closely spaced microvilli, long overlapping cytoplasmic regions with well-defined junctional complexes. The parental line (R3327-5') demonstrated a combi-

nation of both these growth patterns. E-cadherin expression was absent in clones 5'A, 5'C, and 5'D and very prominent in clone 5'B, when compared to the parental line. The absence of E-cadherin expression correlated with increased invasiveness, as measured in an in vitro invasion assay. Subcutaneous injections of clones 5'A, 5'C, and 5'D yielded lung metastases and no primary tumors at the site of inoculation while clone 5'B was tumorigenic and produced fewer lung metastases in vivo. These clones, therefore, provide a potential for studying a variety of molecules involved in prostate cancer invasion and metastasis, especially for the direct testing of the significance of E-cadherin expression in prostate cancer progression. (Pathology Oncology Research Vol 3, No 4, 264-271, 1997)

Key words: prostate cancer, invasion, metastasis, E-cadherin, heterogeneity

Introduction

The Dunning R-3327 rat adenocarcinoma model was derived from a spontaneous rat prostatic cancer in 1963.⁵ The tumor has been maintained by serial transplantation using minced tumors or cells from tissue culture. In the ensuing years, the genetic instability of this tumor has

given rise to more than 20 sublines which have been widely used to study a variety of prostatic tumor biology questions.¹³ The wide spectrum of metastatic potential exhibited by these sublines allows for study of the molecules involved in prostatic tumor cell invasion and metastasis. However, the cellular heterogeneity demonstrated by the Dunning tumor sublines adds a large degree of complexity to this experimental system. The establishment of cell lines derived from single cell clones and the subsequent characterization of the differentially expressed invasive and metastatic properties distinguishing the cloned cell lines will provide useful models to investigate the biological regulation of prostatic tumor cell invasion and metastasis.

In this regard, E-cadherin is a 120-KDa transmembrane glycoprotein that is involved in calcium-dependent cell-cell adhesion.¹⁹ Loss or decreased E-cadherin expression or function correlates with an enhanced aggressiveness and

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dedifferentiation of many carcinomas.²⁰ Subsequent to the initial observation that E-cadherin expression is decreased during the progression of rat prostate cancer using the Dunning model,² numerous studies have indicated that aggressive human prostate tumors exhibit decreased E-cadherin expression.^{3,17,22} The heterogeneity with regard to E-cadherin expression may in fact account for the reduced E-cadherin abundance observed in malignant tumors, both in human and in the current animal models. In order to gain insight into the role of E-cadherin in the progression of prostate cancer, it is necessary to separate the E-cadherin positive and E-cadherin negative cells in an animal model to demonstrate the correlation between loss of E-cadherin expression and an enhancement of metastatic potential.

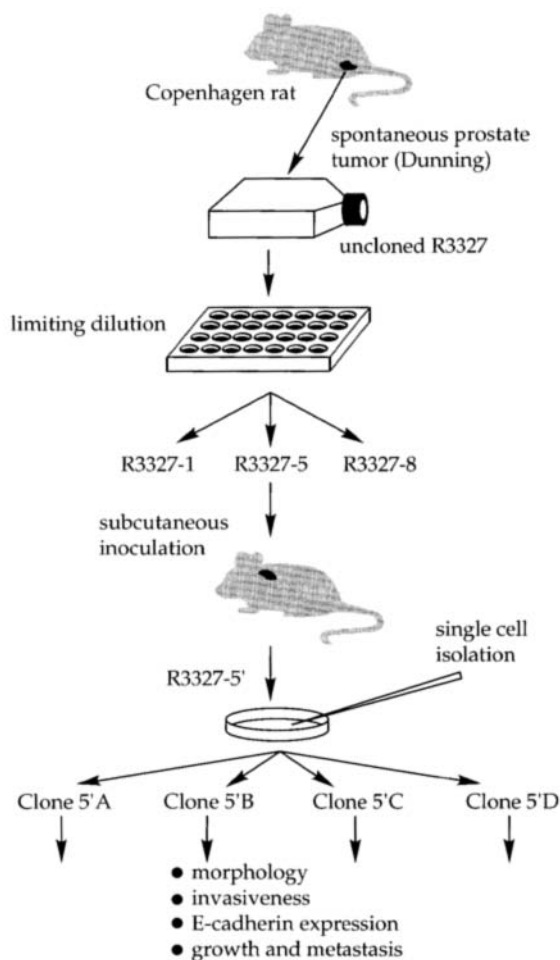


Figure 1. Experimental methodology flow chart. Cultured late passage cells from a spontaneous rat prostate tumor were cloned by a limiting dilution procedure. R3327-5, a low invasive subline *in vitro*, was injected s.c. into the right flank of male Copenhagen rats. Single cells were cloned from the heterogeneous cultures of the excised tumor to yield 4 morphologically distinct clones, which were subsequently assayed for differences in morphology, E-cadherin expression, and invasive and metastatic potential.

Thus, in this study, we capitalized on the cell clones derived by limiting dilution from a heterogeneous Dunning R-3327 tissue culture line, which were previously shown to exhibit different morphologic features.²¹ One of the derivative cell lines (which were poorly invasive) underwent spontaneous progression in the tumor-bearing animals and gave rise to a more invasive, morphologically heterogeneous cell line. Four cell clones were subsequently derived from this parental line based on cell morphology. We then characterized the invasive and metastatic properties and report herein a correlation between loss of E-cadherin expression and gain of invasive and metastatic potential in the tumor lines.

Materials and Methods

Establishment of cell clones

R3327-1, R3327-5 and R3327-8 are three cell lines cloned from the late-passage tissue cultures of a slow-growing, androgen dependent subline of the Dunning tumor R-3327/132 by the limiting dilution method.²¹ Cells were maintained *in vitro* in RPMI 1640 supplemented with 2mM L-glutamine (Gibco, Grand Island, NY), 10% fetal bovine serum (Gibco), 1mM sodium pyruvate (Gibco), 10 mM HEPES (Gibco), 100 units/ml penicillin-streptomycin sulfate (Gibco), and 0.2% gentamycin (Sigma, St. Louis, MO). A 1 ml cell suspension containing 3×10^6 cells was inoculated s.c. into 8-week-old male Copenhagen rats (Harlan Sprague-Dawley, Indianapolis, IN). To establish primary cultures of the subcutaneous tumor, rats were sacrificed and 1 mm³ sections of viable tumor were excised and mechanically disrupted under sterile condition. The resulting cell lines, designated R3327-1', R3327-5' and R3327-8', were then maintained under the same conditions as the original cells before inoculation. R3327-5' cells exhibited cellular heterogeneity characterized by two distinctive morphologies. To generate clonal cell lines from R3327-5', single cells were selected from a trypsinized culture using a micropipette with a polished tip and transferred to 96-well tissue culture plates. Four cell clones were established based on cell morphology and designated clone 5'A, clone 5'B, clone 5'C, and clone 5'D.

Morphological studies

The clones were maintained under the conditions described above. Phase contrast pictures were taken when the cells approximated 70% confluence to record the distinctive morphologies. For transmission electron microscopy (TEM) and scanning electron microscopy (SEM) analysis, cells (passage 4) were grown in 24-well tissue culture plates to confluence. The cells were then

fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer and processed for SEM and TEM, as has been described in detail.^{18,21}

Invasion assay

The Membrane Invasion Culture System (MICS) assay was performed as previously described.¹⁰ Briefly, a polycarbonate membrane with 10 micron pores (Osmonics, Livermore, CA) was coated with a uniform thickness of defined basement membrane matrix containing laminin/type IV collagen/gelatin and placed inside the MICS chambers (which are modified Boyden chambers). Both the upper and lower chambers were filled with RPMI 1640 Mito+™ serum free medium (Collaborative Research, Bedford, MA). Cells were seeded at a concentration of 1×10^5 cells/well. After incubation for 24 hours the cells that had invaded the basement membrane matrix were collected and stained for counting. Invasion rate was calculated as the percentage of cells capable of invading the membrane compared to the total number of cells seeded.¹⁰

In vivo growth and metastasis assay

To assay for the metastatic potential and tumorigenicity of the cell clones, 1×10^6 cells were injected subcutaneously in the right flank of 8-week-old Copenhagen rats (Harlan Sprague-Dawley). Seven rats were injected for each cell line. All the rats were sacrificed after one month post-inoculation. Tumors at the site of injection were measured with a caliper, and lung metastases were counted 12 hours after fixation with 25% Bouins solution in 3.7% formaldehyde.

Immunological detection of E-cadherin

For indirect immunostaining, cells were grown on glass coverslips in 24-well tissue culture plates to confluence, washed with PBS three times, and fixed in 3.7% formaldehyde for 7 minutes. After extensive washing the coverslips were blocked for 30 minutes with PBS containing 1% BSA and 0.1% Triton X-100. The cells were then incubated with anti-E-cadherin monoclonal antibody (Transduction Laboratories, Lexington, KY) diluted 1:100/PBS. Coverslips were washed further and incubated for 30 minutes with rhodamine-conjugated goat anti-mouse secondary antibody (Zymed, San Francisco, CA) diluted 1:50/PBS. After additional washing, coverslips were mounted in gelvatol and visualized using a Zeiss fluorescence microscope.

For immunoblot analysis, cells were grown to confluence and treated with lysis buffer containing 125 mM Tris-HCl, pH 6.8, 2% SDS, 5% glycerol, 0.003% bromophenol blue and 1% beta-mecaptoethanol. The protein

concentration was measured by dotMETRIC protein quantification kit (GenoTechnology, St Louis, MO). 20 µg protein/lane were separated by 10% SDS-PAGE and transblotted to Immobilon-P membrane (Millipore,

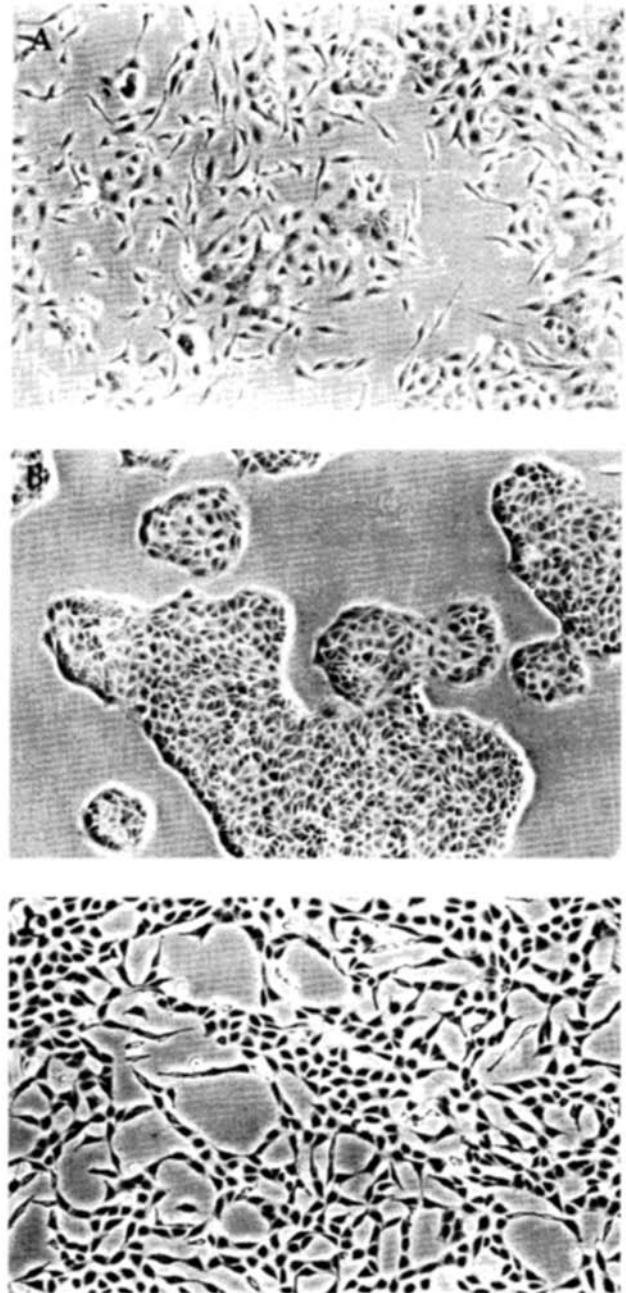


Figure 2. Light micrographs of cell cultures: (a) The parental R3327-5'; (b) clone 5'B; and (c) clone 5'C. These cultures exhibited distinctive morphologies. Note the sparse, spindle-shaped cells mingled with polygonal cells in the parental culture; the islands of closely adherent polygonal cells in clone 5'B; and the interlacing pattern of elongate cells characteristic of clone 5'C cultures. Clones 5'A and 5'D displayed similar morphological features as clone 5'C. Magnification=220x

Bedford, MA). Membranes were blocked by incubation with washing buffer containing 5% nonfat milk and 1% BSA overnight and then incubated for one hour at room temperature with anti-E-cadherin monoclonal antibody, diluted to 1:2500/PBS. Blots were rinsed with washing

buffer and incubated with HRP-conjugated goat anti-mouse IgG, diluted 1:5000/PBS (American Qualex, LA Mirada, CA) for one hour and, after 5 washes, visualized by ECL Western blot detection kit (Amersham, Buckinghamshire, England).

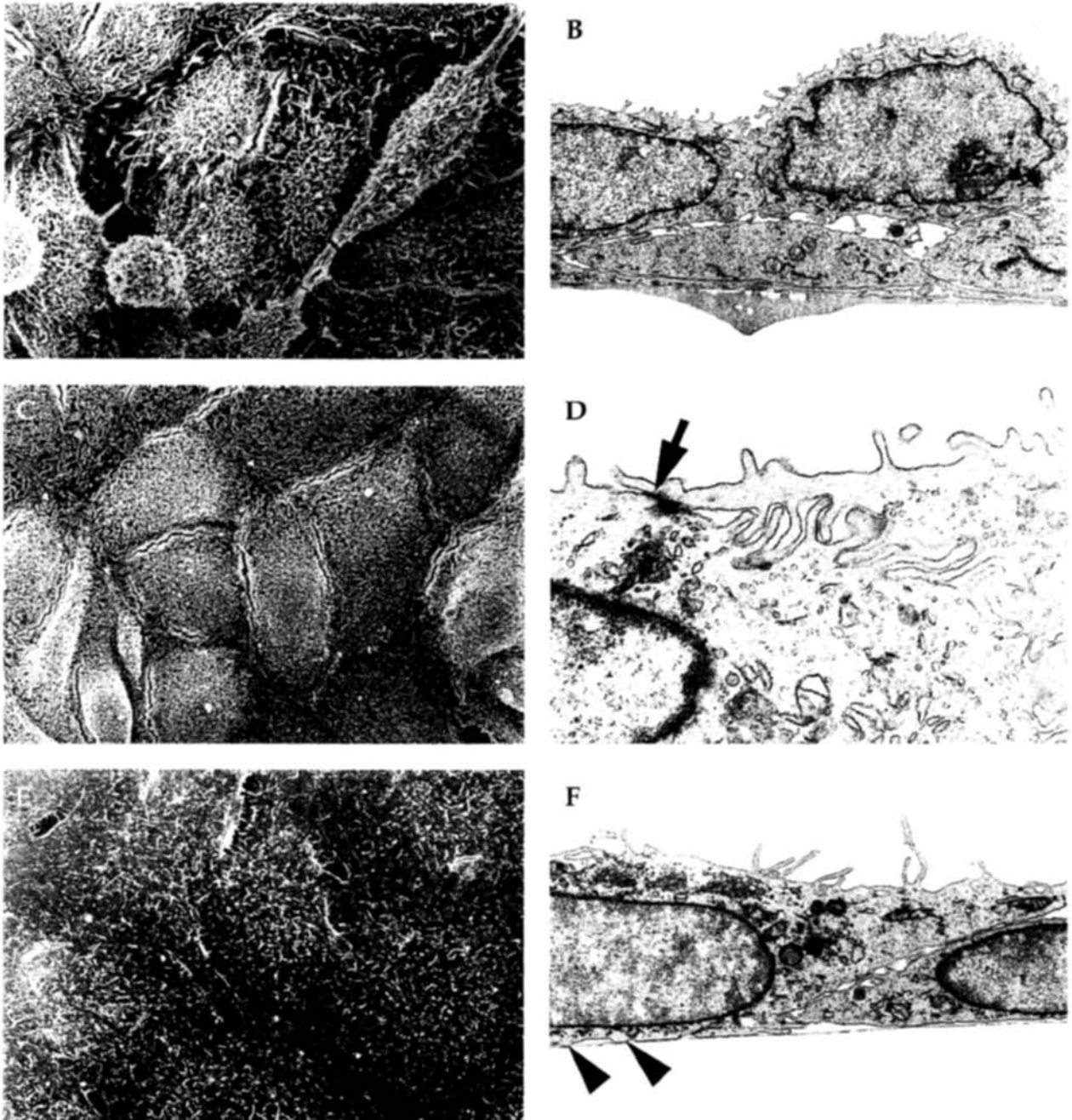


Figure 3. Scanning electron micrographs (a, c, e) and transmission electron micrographs (b, d, f) of the cell cultures showing the distinctive cell surface projections and junctional areas in the parental R3327-5' (a, b), clone 5'B (c, d), and clone 5'C (e, f) cells. Note the diversity of cellular morphologies and surface modifications present in the parental R3327-5' cells (a, b); the junctional complex (arrow) in clone 5'B (d); and the punctate cell-substratum adhesions (arrowheads) and complex membrane projections (microplacae and branching microvilli) in clone 5'C (e, f). Clones 5'A and 5'D exhibited the same SEM and TEM morphology as clone 5'C (data not shown). Magnification: a=1530x, b=4980x, c=1530x, d=15000x, e=830x, f=7500x.

Results

Morphological characterization of cell clones

The experimental strategy for our study is illustrated in *Figure 1*. R3327-1, R3327-5 and R3327-8, the three cell lines of unicellular origin, demonstrated homogeneous cell morphology at the light microscopic (LM) level prior to inoculation.²¹ When inoculated into Copenhagen rats, these cells formed subcutaneous tumors. The derivative primary cell lines (R3327-1', R3327-5' and R3327-8') developed from the subcutaneous tumors all exhibited heterogeneous cell morphologies, possibly from genetic changes that may have occurred spontaneously during solid tumor growth *in vivo*. This change was best demonstrated in the R3327-5 cells. This poorly invasive and poorly tumorigenic R3327-5 cell line underwent a spontaneous progression *in vivo*, which resulted in highly invasive, highly tumorigenic heterogeneous cells (R3327-5'). There are two basic morphologies illustrated: fibroblastic and compact epithelial (*Figure 2*). From the parental R3327-5' cell line, four cells were selected by use of a hand-pulled glass micropipette and gave rise to four clonal cell lines.

The four cell clones exhibited distinct cellular morphologies at the LM level (*Figure 2*). Three of them (clones 5'A, 5'C, 5'D) demonstrated a fibroblastic morphology; cells were loosely connected to each other. Clone 5'B grew in tight clusters and appeared epithelial-like. The parental R3327-5' exhibited a combination of these growth patterns. Floating cells were consistently observed in clones 5'A, 5'C, and 5'D cell cultures but not in clone 5'B (data not shown). These floating cells were observed to attach to the substratum again after being transferred to another tissue culture well with fresh medium. All the cell lines were maintained for more than 20 passages *in vitro* without microscopically visible morphological changes.

Scanning and transmission electron microscopy studies (*Figure 3*) revealed detailed differences in the junctional areas and surface features of cell clones. Clones 5'A, 5'C, and 5'D demonstrated a pattern of complex membrane projections in the form of branching microvilli and microplicae. Long microvilli were frequently observed to bridge the space between closely apposed cells. These features were not seen in clone 5'B. Ultrastructural analysis of clone 5'B showed closely spaced surface microvilli and long overlapping cytoplasmic regions along the cell-cell interface. Junctional complexes were copious. The basal surfaces of the clone 5'B cells established contact with the substrate by means of a uniform, flat region of contact (data not shown), while clones 5'A, 5'C, and 5'D exhibited punctate adhesion regions of the basal cell membrane with the substrate which suggest a loose attachment or affinity for the substrate.

Immunohistochemical analysis of E-cadherin expression

It has been shown that human carcinoma cell lines with an epithelioid morphology are generally E-cadherin positive and non-invasive, whereas cell lines with a fibroblas-

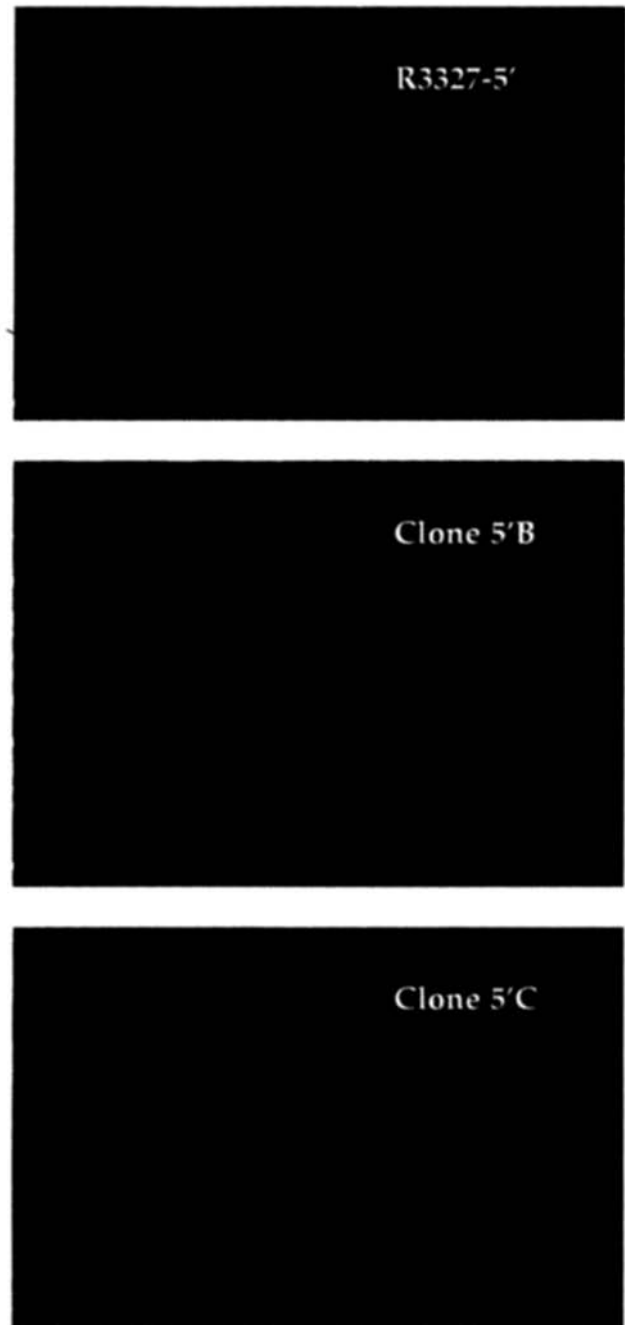


Figure 4. E-cadherin expression in cell cultures by immunofluorescence analysis. Positive staining was only observed in clone 5'B (middle) and in the epithelial clusters in the heterogeneous R3327-5' parental line (top). Clones 5'A and 5'D have the same negative staining pattern as clone 5'C (bottom). Magnification=1400x

toid morphology are generally invasive and lack E-cadherin expression.⁷ As illustrated in *Figure 4*, immunohistochemical staining of E-cadherin was detected uniformly in clone 5'B and in the epithelial clusters in the heterogeneous R3327-5' parental cells. Positive labeling was predominantly distributed along the cell-cell junctional border. Only background E-cadherin staining was found in clones 5'A, 5'C, 5'D and in the fibroblastic cells within the heterogeneous R3327-5' parental cell line, even when cells were allowed to grow to confluence. Controls receiving only secondary antibody showed no staining (data not shown). The consistency of this pattern of E-cadherin expression was confirmed by repeating the immunostaining at different passages.

To validate the immunofluorescence results, western blot analysis was performed (*Figure 5*). Similarly, clone 5'B and the parental R3327-5' both showed a 120 KDa band corresponding to E-cadherin, and only background staining was observed in clones 5'A, 5'C and 5'D. Again, this pattern did not change over *in vitro* passage as confirmed by repeating the western blot analysis at different passages.

Assesment of invasive and metastatic potential

The MICS assay was used to measure the ability of the cloned cells to invade a basement membrane matrix *in vitro*. Invasion rates of 11.3%, 13.5%, 20.1% and 14.1% were observed for R3327-5', clones 5'A, 5'C and 5'D, respectively, whereas only 3.8% of the clone 5'B cells were able to invade (*Figure 6*). These data correlated with the presence of E-cadherin at the junctional complex.

The tumorigenicity and metastatic ability of the cell clones were further tested in the rat model (*Table 1*). Consistent with the *in vitro* invasion data, s.c. inoculation of clones 5'A, 5'C and 5'D produced more lung metastases than that of clone 5'B ($P < 0.025$ when clone 5'B was compared to clones 5'A, 5'C, and 5'D by students T test). Surprisingly, no subcutaneous tumors were found one month following inoculation of clones 5'A, 5'C and 5'D. To test if there was a latent phase for clones 5'A, 5'C and

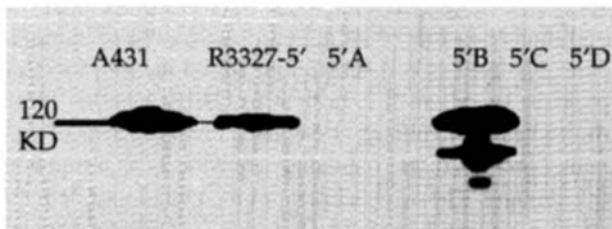


Figure 5. E-cadherin expression in cell cultures by Western Blot analysis. Only clone 5'B and the parental R3327-5' showed a 120 KDa band corresponding to E-cadherin. A431 cell lysate was used as a positive control.

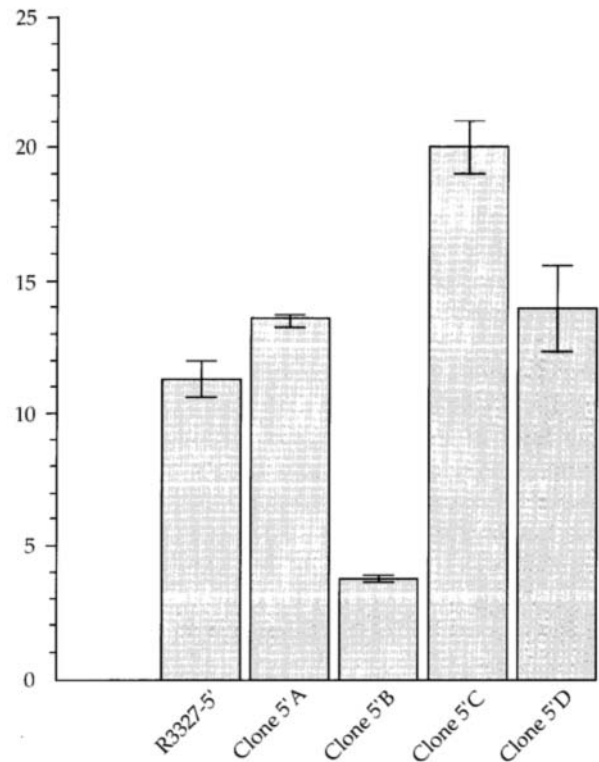


Figure 6. Unstimulated invasive ability of prostatic cell lines as measured using a modified Boyden chamber assay (Membrane Invasion Culture System; MICS chamber). Invasive ability was calculated as the percentage of cells capable of invading a laminin/collagen IV/gelatin-coated polycarbonate membrane over 24 hrs, compared with the total number of cells seeded (\pm SD determined).

5'D to form subcutaneous tumors, another group of rats was sacrificed 75 days after inoculation, and still no tumors were found. By comparison, inoculation of clone 5'B produced smaller subcutaneous tumors in the rat than the parental R3327-5'.

Discussion

Cellular heterogeneity is a fundamental characteristic of neoplasia. Tumors are composed of subpopulations of cells with different biological properties that result from spontaneous genetic changes.⁶ The Dunning R3327 rat prostatic adenocarcinoma is an established animal model for the study of the human form of prostatic malignancy. Cellular heterogeneity is well demonstrated in this system in that many sublines have been developed by selection for certain biological properties such as androgen responsiveness, degree of differentiation, tumor growth, and the ability to metastasize.^{11,13} Recently, several key regulators of prostate tumor invasion and metastasis have been characterized using this model system.^{1,4,9} However, no attempt has been made to generate clones *in vitro* with

distinctive cellular morphologies to demonstrate the heterogeneous expression of invasive and metastatic properties and key markers involved in prostate tumor progression. The present study focused upon the spontaneous progression that occurred in the rat model inoculated with R3327-5 cells, with subsequent ability to establish heterogeneous primary cultures composed of morphologically distinguishable subpopulations of cells. Four clones have been derived from this primary culture, which demonstrated two disparate biological characteristics. Three of the four clones (clones 5'A, 5'C, and 5'D) demonstrated a fibroblastic morphology and were negative for E-cadherin expression. This correlated with a high level of invasiveness and metastatic ability. The fourth clone (clone 5'B) grew in tight clusters and was positive for E-cadherin expression, which was associated with low invasive and metastatic potential. The distinctive features exhibited by the cell clones can be maintained during long term *in vitro* culture.

The original R3327-5 cell line was an established cell line of single cell origin.²¹ It was E-cadherin positive and exhibited low invasive potential *in vitro* before inoculation (data not shown). Upon injection of cells into Copenhagen rats, the developed tumors demonstrated heterogeneous E-cadherin expression as a result of loss of this proteins expression in a subpopulation of cells, as well as higher invasive and metastatic potentials. This is in agreement with previously reported observations that heterogeneous expression of E-cadherin is related to invasive behavior.² In this study, E-cadherin positive and E-cadherin negative cells were cloned, and the invasiveness of the cloned cells was consistently correlated with loss of E-cadherin expression.

We further observed in this model that although the E-cadherin negative clones (clones 5'A, 5'C and 5'D) were highly metastatic and formed foci in the lungs, they did not produce noticeable primary tumor at the site of injection. The mechanism of the failure of the E-cadherin negative cells to initiate solid tumor growth is not known. It is possible that the majority of the injected cells migrated away from the injection site, and the remaining cells failed to overcome the host pressure to form solid tumor. Despite its very low invasiveness, clone 5'B also metastasized to lung (though significantly less than other clones). This phenomenon reflects the complex nature of *in vivo* metastasis. We had speculated that clone 5'B might become heterogeneous upon inoculation, similar to the results that were obtained when the original R3327-5 cells were injected. However, we found that unlike the R3327-5 cells, clone 5'B cells maintained their compact epithelial morphology *in vivo* (data not shown); this suggests there are other mechanisms which enable the clone 5'B to metastasize, other than change in E-cadherin expression.

Table 1. Tumorigenic and metastatic potential of the clones

Cell lines	Primary tumor growth	Metastatic ability ^a (no. of lung metastases/animal)
R3327-5'	++	63 ± 13
Clone 5'A	-	81 ± 7 ^b
Clone 5'B	+	46 ± 10
Clone 5'C	-	80 ± 11 ^b
Clone 5'D	-	86 ± 7 ^b

^a Data collected at 28 days after s.c. inoculation. n = 7.

^b P < 0.025 when compared to clone 5'B by students' T test

This model offers the opportunity for studying a variety of markers important to the metastatic phenotype in prostatic adenocarcinoma. Several molecular prognostic markers for the metastatic potential of diagnosed human prostate tumors have been reported.¹⁶ E-cadherin is one such marker which has been extensively studied.^{3,17,22} Loss or decreased function of E-cadherin has been observed in nearly half of all prostate cancers and is believed to be a critical step for the acquisition of metastatic potential of aggressive prostate cancers.¹² The mechanism by which loss of E-cadherin may promote prostate cancer metastasis requires full investigation. Studies have indicated that loss of E-cadherin activities may have consequences other than loss of cell adhesion. Frixen and colleagues have shown that abrogation of E-cadherin function leads to up-regulation of mRNA and protein synthesis of an extracellular matrix degrading protease, urokinase-type plasminogen activator,⁸ suggesting that loss of E-cadherin function may direct a signaling pathway which stimulates expression of the matrix-degrading enzyme. Indeed, E-cadherin is not the only molecule which is functionally altered during cancer progression. Other molecules whose function are altered accompanying the loss of E-cadherin include autocrine motility factor receptor (gp78),¹⁵ fibronectin and its receptor ($\alpha 5\beta 1$).¹⁴ The floating cells observed in the cell cultures and the loose attachment to the substrate revealed by electron microscopy of E-cadherin negative cells also suggested that there may be other changes accompanying loss of E-cadherin, such as those in molecules involved in cell-substratum adhesion, apoptosis, or protease secretion. It is critical to clarify whether these molecules are regulated at the same level in terms of signaling process or are regulated sequentially as a function of signaling. The establishment of the cell clones demonstrating inverse correlation between E-cadherin expression and metastasis in this model will allow for the direct testing of the significance of E-cadherin in prostate cancer invasion and metastasis. Future studies utilizing this model will focus on these perspectives.

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References

1. Bao L, Loda M, Janney PA *et al*: Thymosin beta 15: A novel regulator of tumor cell motility upregulated in metastatic prostate cancer. *Nature Medicine* 2(12):1322-1328, 1996.
2. Bussemakers MJG, Moorselaar RJA, Girolodi LA *et al*: Decreased expression of E-cadherin in the progression of rat prostate cancer. *Cancer Research* 52:2916-2922, 1992.
3. Cheng L, Nagabhushan M, Pretlow TP *et al*: Expression of E-cadherin in primary and metastatic prostate cancer. *American Journal of Pathology* 148(5): 1375-1380, 1996.
4. Dong JT, Lamb PV, Rinker-Schaeffer CW *et al*: KAI1, a metastasis suppressor gene for prostate cancer on human chromosome 11p11.2. *Science* 268:884-886, 1995.
5. Dunning WF: Prostate cancer in the rat. *Monographs of the National Cancer Institute* 12:351-369, 1963.
6. Fidler IJ, Hart IR: Biological diversity in metastatic neoplasms: origins and implications. *Science* 217:998-1003, 1982.
7. Frixen U, Behrens J, Sachs M *et al*: E-cadherin mediated cell-cell adhesion prevents invasiveness of human carcinoma cells. *Journal of Cell Biology* 111:173-185, 1990.
8. Frixen UH, and Nagamine Y: Stimulation of urokinase-type plasminogen activator expression by blockage of E-cadherin-dependent cell-cell adhesion. *Cancer Research* 53:3618-3623, 1993.
9. Gao AC, Lou W, Dong JT, and Isaacs JT: CD44 is a metastasis suppressor gene for prostate cancer located on human chromosome 11p13. *Cancer Research* 57:846-849, 1997.
10. Hendrix MJC, Seftor EA, Seftor REB and Fidler IJ: A simple quantitative assay for studying the invasive potential of high and low metastatic variants. *Cancer Letters* 38:137-147, 1987.
11. Isaacs JT, Wake N, Coffey DS, and Sandberg AA: Genetic instability coupled to clonal selection as a mechanism for tumor progression in the Dunning R-3327 rat prostatic adenocarcinoma system. *Cancer Research* 42:2353-2361, 1982.
12. Isaacs WB, Bova GS, Morton RA *et al*: Molecular biology of prostate cancer progression. *Cancer Surveys* 23:19-32, 1995.
13. Isaacs JT: The R-3327 System of rat prostatic cancers. *Urologic Oncology* 2(4): 115-116, 1996.
14. MacCallman CD, Brodt P, Doublet JD *et al*: The loss of E-cadherin mRNA transcripts in rat prostatic tumors is accompanied by increased expression of mRNA transcripts encoding fibronectin and its receptor. *Clinical & Experimental Metastasis* 12(2):101-107, 1994.
15. Otto T, Birchmier W, Schmidt U *et al*: Inverse relation of E-cadherin and autocrine motility factor receptor expression as a prognostic factor in patients with bladder carcinomas. *Cancer Research* 54(12):3120-3123, 1994.
16. Rinker-Schaeffer CW, Partin AW *et al*: Molecular and cellular changes associated with the acquisition of metastatic ability by prostatic cancer cells. *Prostate* 25:249-265, 1994.
17. Ross JS, Figge HL, Bui HX *et al*: E-cadherin expression in prostatic carcinoma biopsies: Correlation with tumor grade, DNA content, pathological stage, and clinical outcome. *Modern Pathology* 7(8): 835-841, 1994.
18. Rowley DR, Feuchter FA, and Heidger PM: Aclar plastic substratum: A cell culture method facilitating preparations for electron microscopy and replicate sampling. *Tissue Culture Assn. Man.* 5:1067-1069, 1979.
19. Takeichi M: Cadherins: a molecular family important in selective cell-cell adhesion. *Annual Review of Biochemistry* 59: 237-252, 1990.
20. Takeichi M: Cadherins in Cancer: implications for invasion and metastasis. *Current Opinion in Cell Biology* 5:806-811, 1993.
21. Thompson SA, Johnson MP, Heidger PM and Lubaroff DM: Characterization of the heterogeneity of R-3327 rat prostatic tumors derived from single-cell clones. *The Prostate* 6:369-387, 1985.
22. Umbas R, Isaacs WB, Bringuier PP *et al*: Decreased E-cadherin expression is associated with poor prognosis in patients with prostate cancer. *Cancer Research* 54(14):3929-3933, 1994.