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Induction of human Cdc37 in prostate cancer correlates with the ability of targeted Cdc37 expression to promote prostatic hyperplasia

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The Cdc37 gene encodes a 50 kDa protein which targets intrinsically unstable oncoprotein kinases such as Cdk4, Raf-1, and src to the molecular chaperone Hsp90. This activity is thought to play an important role in the establishment of signaling pathways controlling cell proliferation. The budding yeast Cdc37 homolog is required for cell division and mammalian Cdc37 is expressed in proliferative zones during embryonic development and in adult tissues, consistent with a positive role in proliferation. Here we report that human prostatic tumors, neoplasias and certain pre-malignant lesions display increased Cdc37 expression, suggesting an important and early role for Cdc37 in prostatic transformation. To test the consequences of increased Cdc37 levels, transgenic mice expressing Cdc37 in the prostate were generated. These mice displayed a wide range of growth-related abnormalities including prostatic epithelial cell hyperplasia and dysplasia. These data suggest that the expression of Cdc37 may promote inappropriate proliferation and may be an important early step in the development of human prostate cancer. *Oncogene* (2000) **19,** 2186–2193.

Keywords: Cdc37; cyclin dependent kinase; chaperone; cancer; transgenic mice

Introduction

Benign prostatic hyperplasia (BPH) and prostate cancer are the most common prostate abnormalities affecting older men. These are multifactorial disease processes, which involve biochemical, genetic and epigenetic factors. Their pathogenesis, however, remains poorly understood. Nodular or glandular hyperplasia is diagnosed in about 20% of males by 40 years of age. The frequency of nodular hyperplasia is increased progressively with age, reaching more than 90% of men in their eighties (Coffey, 1992; Walsh, 1992). Prostate carcinoma is the most frequently diagnosed cancer, with incidence approaching 60% in men older than 80 years of age, and is the second most common cause of cancer-related deaths in men. Despite significant advances in understanding and treating cancer in general, the mortality resulting from prostate cancer has remained high (Landis *et al*., 1999).

Prostate cancer is a complex disease in which focal transformation occurs with frequent and unpredictable metastasis. A variety of mutations have been linked with prostate cancer (reviewed in Thompson *et al*., 1999) and many of these mutations impinge on some aspect of cell cycle control. The decision to enter the cell division cycle is made during G1 (first gap phase), a period where cells are responsive to extracellular growth signals. In response to net positive signals, cells activate signal transduction pathways that culminate in the expression genes required for cell cycle progression. The *ras* signaling pathway is a central component of this transduction cascade and has been shown to be required for the induction of cyclin D1 expression (Aktas *et al*., 1997; Peeper *et al*., 1997). D-type cyclins function as regulatory subunits of Cdk4 and Cdk6 and these complexes are required for the G1-S transition. Because the levels of D-type cyclins are sensitive to the presence of mitogens, they function as sensors to control the decision to enter S phase. Cdk4 and Cdk6 have been implicated in the inactivation of Rb, leading to activation of E2F-dependent transcriptional programs, and in titration of the Cdk2 inhibitor $p27^{KIP1}$, leading to activation of cyclin E/Cdk2 which is also required for S phase. (Ewen *et al*., 1993; Kato *et al*., 1993, 1994; Matsushime *et al*., 1994; Meyerson and Harlow, 1994; Sherr and Roberts, 1999; Reynisdottir *et al*., 1995; Connell-Crowley *et al*., 1997; Reynisdottir and Massague, 1997; Cheng *et al*., 1998; McConnell *et al*., 1999). Assembly of an active Cdk4/cyclin D complex is a multistep process involving at least one mitogen-dependent step (Matsushime *et al*., 1994; Meyerson and Harlow, 1994; LaBaer *et al*., 1997; Cheng *et al*., 1998, 1999). Newly synthesized Cdk4 is assembled into a Cdc37/Hsp90 chaperone complex for stabilization (Dai *et al*., 1996; Stepanova *et al*., 1996; Lamphere *et al*., 1997). Stabilized Cdk4 is apparently then released in a still uncharacterized step, thereby allowing assembly with either regulatory subunits such as cyclin D or with inhibitors such as p16 (reviewed in Sherr and Roberts, 1999).

Previously, we demonstrated that the Cdc37 gene encodes the Hsp90-associated p50 protein (Stepanova *et al*., 1996). p50 was previously seen in complexes with several kinases implicated in mitogenic signaling pathways, including v-*src* (Brugge, 1981, 1986; Whitelaw *et al*., 1991; Hutchison *et al*., 1992); *src* homologs (Brugge, 1986) and Raf (Stancato *et al*., 1993), but its identity was unknown. Genetic and biochemical

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data in several systems suggests that particular protein kinases are intrinsically unstable and their association with the Cdc37/Hsp90 chaperone in the cytoplasm is important for folding and/or activation of the targeted kinase (Cutforth and Rubin, 1994; Gerber *et al*., 1995; Stepanova *et al*., 1996; Grammatikakis *et al*., 1999; Munoz and Jimenez, 1999; Xu *et al*., 1999). Indeed, Cdc37 is an essential gene in both budding yeast and Drosophila and this is thought to reflect its role in stabilizing growth promoting kinases. Consistent with its role in promoting cell division, Cdc37 is expressed in proliferating zones during mouse development and in adult tissues but is absent from many resting cells (Stepanova *et al*., 1996).

In this study, we have examined Cdc37 expression in normal and transformed human prostate tissues. We found that while Cdc37 protein is low or absent from normal prostatic epithelia, it is highly expressed in transformed epithelium. The presence of Cdc37 expression in some pre-malignant prostatic lesions indicates that the induction of Cdc37 expression might be an early event in the transformation process. To test the effect of the expression of Cdc37 on cell division in the prostate, we constructed two lines of transgenic mice expressing Cdc37 under control of a prostate-specific probasin promoter. As in human tissue, Cdc37 is absence from adult mouse prostatic epithelium. Interestingly, targeted expression of Cdc37 in the mouse prostate resulted in epithelial hyperplasia and dysplasia, and other growth-related abnormalities. In a parallel study, we found that MMTV-Cdc37 caused transformation of mammary epithelium and collaborated with c-*myc* and cyclin D1 to transform multiple tissues (Stepanova *et al*., 2000). These data are consistent with a positive role for Cdc37 in promoting not only normal proliferation but also inappropriate proliferation such as that involved in transformation, and suggests that in some contexts, Cdc37 may be rate-limiting for transformation.

Results

Cdc37 is expressed in premalignant and malignant human prostate epithelia

The expression of Cdc37 mRNA in the developing mouse embryo is tightly correlated with proliferative zones, and is coincident with the cyclin D1 expression

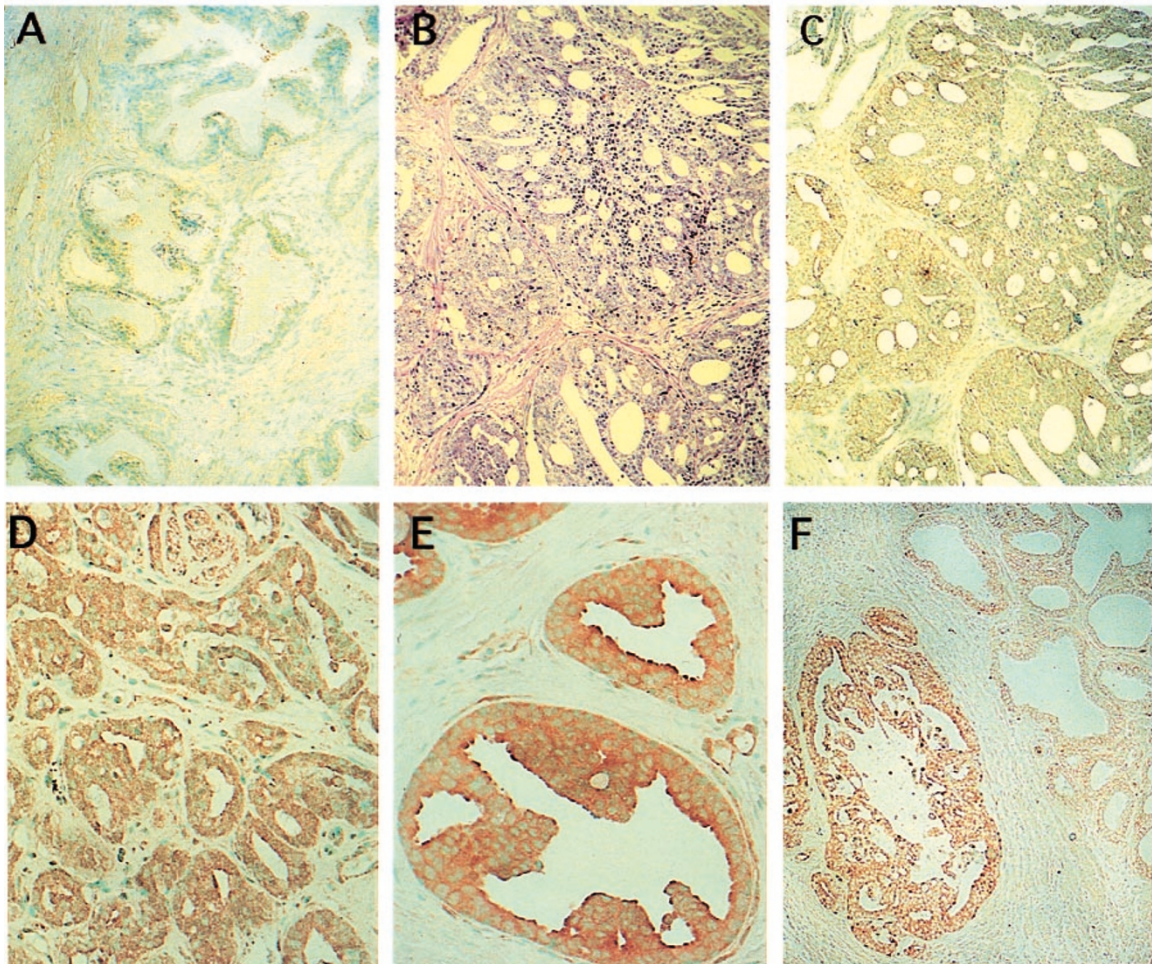

Figure 1 Cdc37 is expressed in the transformed epithelia of human prostate. (**a**) A section from a normal prostate was stained with affinity purified anti-Cdc37 antibodies. Normal epithelial and stromal compartments were typically negative for Cdc37 protein. (**b** and **c**) Adjacent sections of human prostates removed via radical prostatectomy were stained with either (B) H&E or with (**c**) anti-Cdc37 antibodies. Focal cancer are shown and Cdc37 expression is clearly observed in cancer foci. (**d**) High-magnification image of prostate cancer stained for Cdc37 expression. Cdc37 is present in the cytoplasm of a large fraction of epithelial cells in the cancer. (**e**) Cdc37 expression in a PIN-like lesion. Original magnification: **a**–**c** 100×; **d**, 200×; **e**, 400×. (**f**) A section of human prostate containing a transformed foci adjacent to largely normal galandular epithelium stained with anti-Cdc37 antibodies (100× magnification)

in certain tissues (Stepanova *et al*., 1996). Moreover, expression of Cdc37 in the mouse breast epithelium via the MMTV promoter results in development of mammary adenocarcinomas (Stepanova *et al*., 2000). These observations are consistent with the proposed role of Cdc37 in the establishment and maintenance of the growth promoting pathways in normal and transformed tissues.

To evaluate Cdc37 expression in the human prostate in normal and transformed states, we analysed tissue sections for Cdc37 expression using affinity purified rabbit polyclonal antibodies (Stepanova *et al*., 1996). These antibodies recognize a 50 kDa protein in immunoblots of mouse and human fibroblast extracts and immunoprecipitate Cdc37/Cdk4/Hsp90 complexes (Stepanova *et al*., 1996). These antibodies also specifically stain the cytoplasm of fibroblasts where Cdc37 is co-localized with Hsp90. Although weak anti-Cdc37 immunoreactivity was seen in a small proportion of glandular cells and in rare basal cells, most cells in normal prostate did not contain detectable Cdc37 protein when compared to control sections probed with normal rabbit serum (Figure 1a and data not shown). In contrast, elevated Cdc37 levels were found both in cancer cells as well in some pre-malignant lesions (Figure 1b–e). The increased expression of Cdc37 in transformed tissue is readily seen in sections where normal tissue lies adjacent to malignant foci (Figure 1f). To validate the results of immunohistochemistry, immunoblotting of normal and transformed human prostate tissue was performed. Levels of Cdc37 were low in normal tissues (Figure 2a, lanes 3–5). In contrast, levels of Cdc37 in multiple tumors were significantly higher than in normal tissues (lanes 6–8) and were comparable to levels found in human prostate cancer cell lines (DU145 and PC3) (lanes 1 and 2).

All 31 prostate carcinomas analysed exhibited remarkably elevated Cdc37 immunoreactivity, although the proportion of the cells expressing Cdc37 varied among tumors from less than 25% to almost 100% (Figures 1d and 2b). On average, about half of the tumors exhibited high levels of Cdc37 in greater than 50% of tumor cells. Quantitatively, the average Cdc37 staining score in cancers ($n=31$) was significantly higher than in the histologically normal prostate specimens ($n=15$, $P=0.0005$, Mann–Whitney test). Interestingly, however, we did not find a significant correlation between Cdc37 staining and the Gleason scores ($P=0.54$, Kruskal–Wallis test) (Figure 2b). Instead, the highest percentages of Cdc37-positive cells was found in moderately differentiated (Gleason score = 5–7) prostate cancer cells.

We analysed Cdc37 expression in pre-malignant lesion. Among the six high grade prostatic intraepithe-

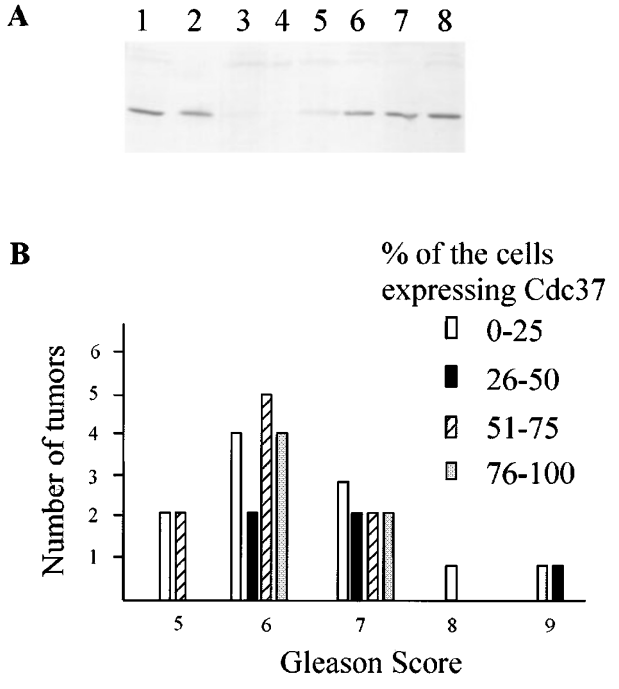

Figure 2 (**a**) Immunoblot analysis of Cdc37 in normal and transformed prostate tissue extracts. Lysates (80 μg) from DU145 cells (lane 1), PC3 cells (lane 2), three independent samples from normal prostate (lanes 3–5) or three independent prostate cancers (lanes 6–8) were separated by SDS–PAGE and immunoblots probed with anti-Cdc37 antibodies. Detection was accomplished using enhanced chemiluminescence. (**b**) The level of Cdc37 expression does not correlate with the Gleason scores of the tumors. Bars represent number of tumors expressing differential levels of Cdc37 for each of the observed values of Gleason scores. Normal tissues and BPH had staining scores of 1

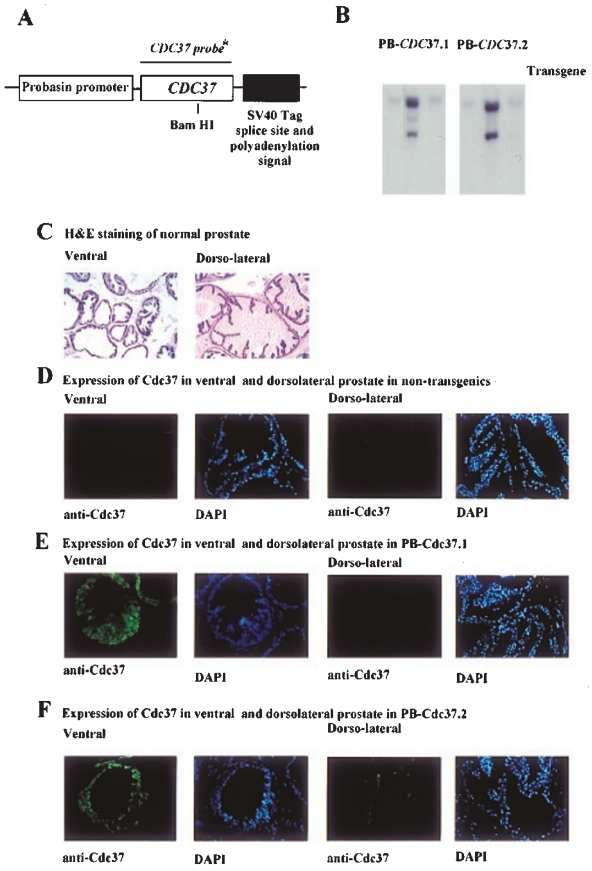

Figure 3 Characterization of PB-Cdc37 transgene expression in the normal prostate of transgenic mice. (**a**) Structure of the construct used to generate PB-Cdc37 mice (see Materials and methods for details). (**b**) Southern blot analysis of PB-Cdc37.1 and PB-Cdc37.2 transgenic lines. Tail DNA was digested with *Bam*HI prior to Southern analysis with a ^{32}P-labeled Cdc37 cDNA probe. (**c–e**) Expression of Cdc37 in the prostate. Sections from control and Cdc37 transgenic mice were subjected to immunofluorescence using anti-Cdc37 antibodies and nuclei were stained with DAPI. Sections from both ventral and dorso-lateral regions are shown. (**d**) specimens from non-transgenic animal, (**e**) specimens from PB-Cdc37.1 animal, (**f**) specimens from PB-Cdc37.2 animal

lial neoplasms (PIN) examined, five showed a remarkably high level of Cdc37 expression (Figure 1e). Occasionally, some prostatic epithelia that were adjacent to PIN or cancer cells and had undergone dysplastic changes also displayed Cdc37 immunoreactivity as well (data not shown). The presence of high levels of Cdc37 in these lesions suggest that induction of Cdc37 expression may be an early step in transformation process.

In marked contrast with tumors, Cdc37 levels in human BPH specimens were low in both stromal and epithelial compartments, with weak staining confined to focal fragments of glandular epithelia with hyperplastic features (data not shown). The average Cdc37 staining score in prostatic epithelia of BPH ($n=8$) was very close to that seen in normal prostatic epithelia ($n=15$) and significantly lower than that in cancer ($n=31$, $P=0.001$, Mann–Whitney test).

PB-Cdc37 transgenic mice

The expression of Cdc37 in early prostatic lesions suggests a possible role in prostatic transformation. To assess the significance of Cdc37 expression in promoting proliferative disorders in the prostate, transgenic mice expressing the mouse Cdc37 cDNA under control

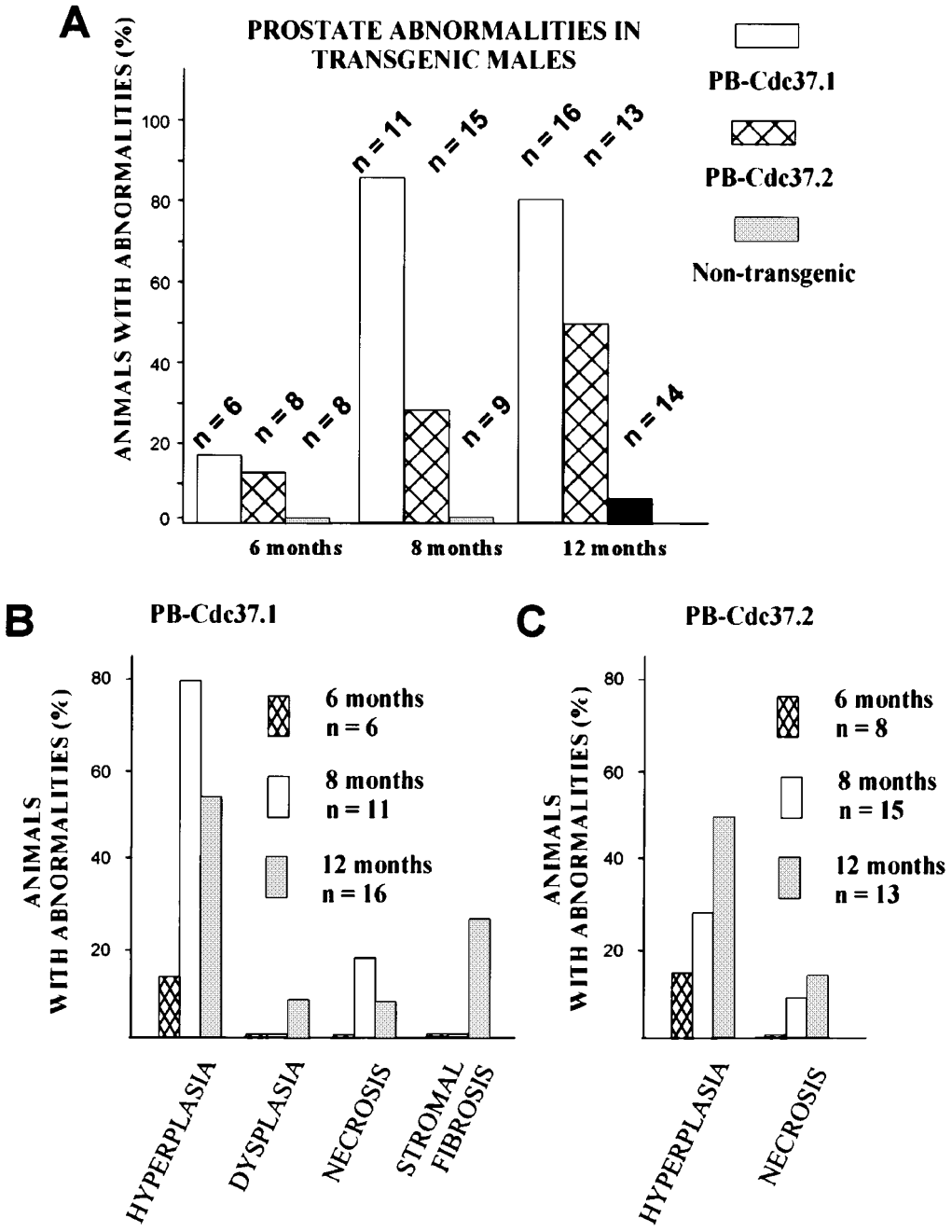

Figure 4 Morphological abnormalities in the prostate of PB-Cdc37 transgenic animals. (**a**) Quantitation of incidence of proliferative disorders at different ages. The percentage of animals with proliferative abnormalities is shown at different ages for control and transgenic males from PB-Cdc37.1 and PB-Cdc37.2 lines. Control animals used in the experiment are non-transgenic littermates of PB-Cdc37.2 males. (**b**) The observed prostatic phenotypes in PB-Cdc37.1 line at different ages. The percentage of the animals developing each phenotype is shown. Some of the animals developed more than one phenotype. (**c**) The observed prostatic phenotypes in PB-Cdc37.2 line at different ages. The percentage of the animals developing each phenotype is shown

of probasin (PB) promoter were generated (Figure 3a). The PB promoter has been shown previously to direct expression of transgenes uniquely to the prostate (Greenberg *et al*., 1995). Two transgenic founders were produced that transmitted the transgene to their progeny and contained multiple copies of the transgene (Figure 3b). Lines of transgenic animals (PB-Cdc37.1 and PB-Cdc37.2) were established by mating each outbred B6D2F1 × ICR founder with outbred ICR mice. The insertion of the transgenic array in the PB-Cdc37.1 line apparently occurred in the Y chromosome, since all male progeny derived from this founder were transgenic while all females were non-transgenic (data not shown). Non-transgenic littermates of the PB-Cdc37.2 line were used as controls for both lines of transgenic animals.

Expression of the Cdc37 transgene was characterized by immunofluorescence of various organs and found, as expected, to be restricted to the male prostate in both lines. Consistent with the results in humans, Cdc37 immunoreactivity was not found in normal mouse prostate epithelium (Figure 3c). In the PB-Cdc37.1 transgenic line, expression was limited to the ventral lobe of the prostate, while both ventral and lateral prostate expressed somewhat lower levels of Cdc37 in the PB-Cdc37.2 line (Figure 3c–e). On average, about 20% of the cells in ventral prostate epithelia of transgenic males in both lines showed the strong cytoplasmic staining for Cdc37, while less than 5% of cell in the dorso-lateral prostate in PB-Cdc37.2 line showed weak expression of Cdc37. Based on immunofluorescence intensity, the level of Cdc37 expression in the ventral prostate epithelium was comparable to the level of Cdc37 observed in tissue culture fibroblasts (data not shown).

Cdc37 expression causes hyperplasia in prostatic epithelium

PB-Cdc37 lines and control littermates were maintained as colonies and monitored for developmental and transformation phenotypes for more than 1 year. Transgenic animals appeared normal during the observation period. Due to the difficulty of observing prostate abnormalities in intact animals, males were sacrificed at 6, 8 and 12 months, and excised prostates examined for proliferative disorders. Growth-related abnormalities in the prostate of rodents are not frequent and their incidence increases with age. The percentage of animals acquiring growth-related abnormalities such as hyperplasia or tumors is strain dependent. On the mixed B6D2F1 × ICR genetic background used for our experiments, less than 10% of males developed some degree of hyperplasia at 12 months of age (Figure 4a). In contrast, prostatic abnormalities in male PB-Cdc37 mice could be seen as early as at 6 months of age (Figure 4a). The number of animals developing prostatic growth-related disturbances increased with age; the percentage of animals developing growth-related abnormalities increased from 18% at 6 months to more than 80% at 8 months of age in the PB-Cdc37.1 transgenic line, and from ~15% at 6 months to ~50% at 12 months of age in the PB-Cdc37.2 line (Figure 4a). The lower proportion of affected animals in the second line may reflect the lower level of Cdc37 expression in that line. All of the abnormalities in the PB-Cdc37.1 line were restricted to the ventral prostate, while both ventral and dorso-lateral prostate was affected in PB-Cdc37.2 line in roughly equal proportion despite the difference in the expression levels between ventral and dorso-lateral lobes (Figure 4 and data not shown).

The most prevalent phenotype observed in both lines was epithelial hyperplasia, which occurred in majority of PB-Cdc37.1 mice and in all PB-Cdc37.2 affected animals (Figure 4b,c). Hyperplasia was frequently accompanied by necrosis. Hyperplasia of the ventral prostate in the PB-Cdc37.1 line was observed as focal lesions involving multiple adjacent acini. Typically, >50% of acini were affected in this strain. The lesions consisted of epithelial proliferation that followed the acinar lining and did not obliterate the lumen (Figure 5a–d). Gradual transitions from completely normal to hyperplastic epithelium were observed (data not shown). Hyperplastic cells retained secretional activity, although usually it was decreased, and the cytoplasmic/nuclear ratio of the cells was

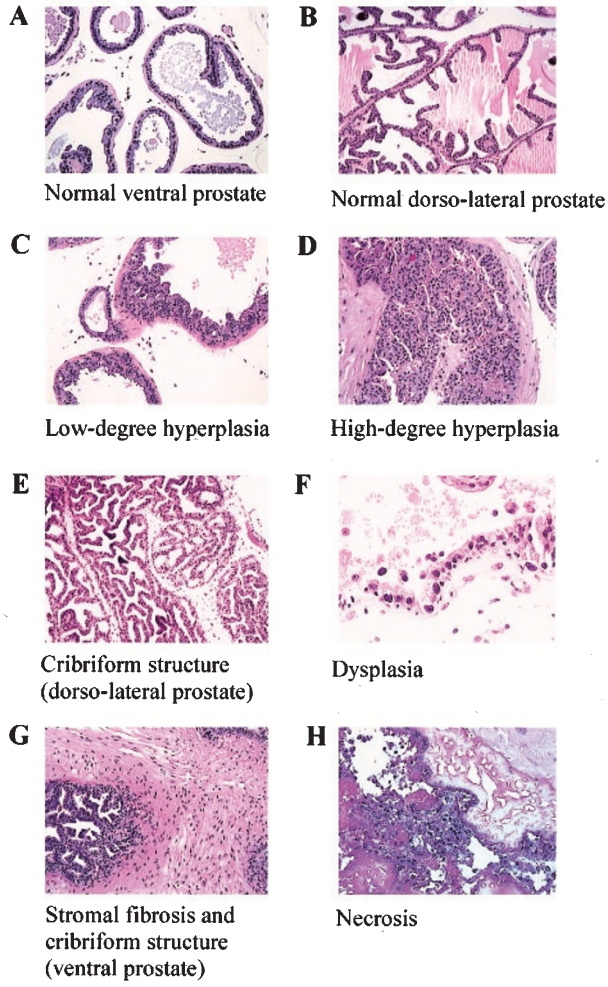

Figure 5 Phenotypes observed in the prostate of PB-Cdc37 transgenic animals. H&E staining of the sections. (**a**) Normal ventral prostate, (**b**) Normal dorso-lateral prostate, (**c**) Low-degree hyperplasia in the ventral prostate of PB-Cdc37.1 male, (**d**) High-degree hyperplasia in the ventral prostate of PB-Cdc37.1 male, (**e**) Cribriform structures formed in the dorso-lateral prostate of PB-Cdc37.2 male, (**f**) Dysplasia in the ventral prostate of PB-Cdc37.1 male, (**g**) Stromal fibrosis in the ventral prostate of PB-Cdc37.1 male adjacent to cribriform structure and (**h**) Necrosis in the ventral prostate of PB-Cdc37.1 male

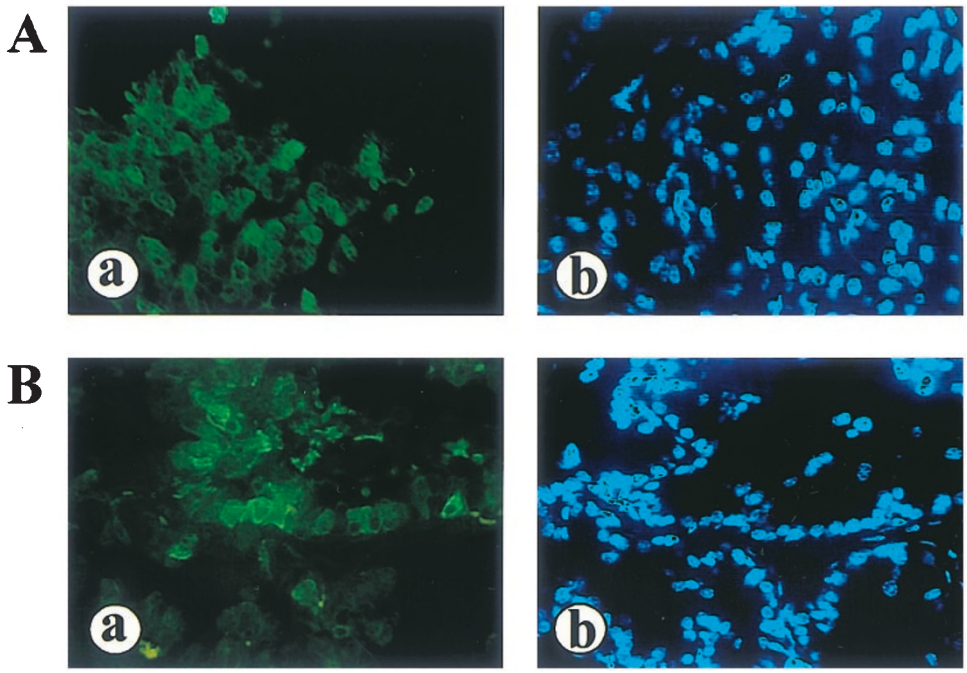

Figure 6 Cdc37 expression in the hyperplastic regions of the transgenic prostate. (**a**–**b**) Cdc37 staining (a) and DAPI nuclear staining (b) in the same section. (**a**) Hyperplasia. (**b**) Cribriform structure

increased in comparison with the normal epithelium. No inflammation was observed in the affected tissues. The development of cribriform structures such as that shown in Figure 5g, adjacent to an area of stromal fibrosis was rare. Cells in hyperplastic areas displayed minimal cellular and nuclear polymorphism. Dysplastic changes were observed in older PB-Cdc37.1 males (Figures 4b and 5f). These changes were focal, affecting usually only several adjacent acini. Single-layered epithelial cells from these regions had irregularly shaped and enlarged nuclei but no mitotic figures were observed. Stromal fibrosis was observed around the areas of high degree hyperplasia, and necrosis was observed in the ventral prostate of the oldest PB-Cdc37.1 males (Figures 4b and 5g,h).

PB-Cdc37.2 males expressed lower levels of Cdc37 in the ventral prostate, and even less in the dorso-lateral lobes. Despite the difference in expression levels between the ventral and dorsal-lateral lobes in this line, the appearance of hyperplasia was observed with equal frequency in these lobes. In both lobes, the most frequent type of the hyperplastic lesions involved cribriform structures (Figure 5e) that completely obliterated the alveolar lumen, although non-cribriform hyperplasia such as observed in the PB-Cdc37.1 line was sometimes observed in the ventral, but not dorso-lateral, prostate. Cribriform hyperplasia was typically multi-focal, affecting several adjacent alveoli. Secretion in affected alveoli was absent, in contrast with normal alveoli, and some necrosis was present in about 30% of the prostates of affected males (Figure 4c).

In both lines of transgenic animals, the abnormalities were observed only in the lobes of the prostate where Cdc37 expression was detected by immunohistochemistry (Figure 3), which correlates with the phenotype being a direct consequence of Cdc37 expression. In addition, Cdc37 expression was found throughout the affected tissue by immunofluorescence (Figure 6).

Discussion

Proliferation in a multicellular organism is a complex process requiring coordination of multiple signaling pathways that link extracellular environmental signals with the cell cycle machinery. Recent studies suggest that Cdc37/Hsp90 complexes are required for the establishment and maintenance of the signaling pathways involving kinases such as *src*, Raf-1 and Cdk4. The interaction of these intrinsically unstable kinases with the Cdc37/Hsp90 complex is required for kinase stabilization and/or activation. Since Cdc37 is required for proliferation in the variety of experimental systems, we examined Cdc37 expression in normal human prostate and in prostate cancer. Cdc37 was found to be largely absent from normal prostate epithelium but was highly expressed in all prostate tumor specimens examined, supporting the idea that Cdc37 plays an important role in proliferation. Interestingly, the highest percentages of Cdc37-positive cells were detected in moderately differentiated (Gleason score 5–7) prostate cancer. Moreover, Cdc37 was highly expressed in the majority of high-grade prostate intraepithelial neoplasias (PIN) (Figures 1 and 2). These observations suggest that the expression of Cdc37 could be an early event in the process of prostatic transformation. These results may mean that proliferative functions are selected for to a greater extent in relatively early disease, whereas prostate cancer cells, with greater metastatic potential, select for other functional activities (Bangma *et al*., 1999). Further studies are required to elucidate the mechanisms by which Cdc37 expression is inappropriately induced during transformation.

To examine the consequences of Cdc37 expression in the prostate, we generated transgenic mice expressing Cdc37 in the prostate epithelium (Figure 3). The majority of males in two independently established transgenic lines displayed dramatic proliferative disorders in the prostate, including epithelial hyperplasia

and dysplasia not observed in control animals (Figures 4–6). Although we cannot absolutely rule out a contribution of other events in the phenotypes observed, the finding that >50% of individual acini in PB-Cdc37 animals are affected suggests that the observed phenotypes are largely, if not exclusively, a result of Cdc37 expression. The rodent prostate was previously noted for its remarkable resistance to overt transformation, and several known single oncogenes are unable to transform the prostate in transgenic models (Sharma and Schreiber-Agus, 1999). There are exceptions to this, however, as polyoma middle T and SV40 large T antigen, both of which have multiple cellular targets, give rise to prostatic transformation in transgenic animals (Sharma and Schreiber-Agus, 1999). In contrast, recent experiments have shown that MMTV-Cdc37 can lead to overt transformation in the mammary gland, although long latency (~20 months) suggests that additional genetic events are important for transformation (Stepanova *et al*., 2000). Interestingly, the extent and rate of transformation in MMTV-Cdc37 animals were greatly enhanced in mice that also expressed either c-*myc* or cyclin D1. In the case of c-*myc*, collaboration with Cdc37 was also observed in the salivary gland and in the Leydig cells of the testis. It will, therefore, be important to determine whether other oncogenes can collaborate with Cdc37 to transform the prostate.

Although the phenotypic consequences of Cdc37 expression in the prostate are striking, the biochemical mechanisms underlying its action are likely to be complex, possibly involving multiple kinase pathways that function interdependently to promote proliferation and possibly other activities consistent with malignancy. Stabilization and/or activation of Cdk4 or Raf could result in both activation of the *ras* pathway and activation of Cdks. In the later case, increased Cdk4 levels could simultaneously sequester $p16^{INK4a}$ and promote proliferation via activation by cyclin D1. This could, in turn, lead to activation of cyclin E/Cdk2 by both increasing cyclin E expression and by sequestration of p27. Interestingly, previous studies have found evidence for increased levels of D-type cyclins in prostate cancer (Aaltomaa *et al*., 1999; Bubendorf *et al*., 1999; Han *et al*., 1998; Kallakury *et al*., 1997) and the available data would indicate that induction of Cdc37 in tumor cells is required to support Cdk4 stability and facilitate formation of active Cdk4/cyclin D complexes that promote proliferation. The induction of components of the cyclin D pathway in prostate cancer is consistent with the low frequency of loss of the Rb tumor suppressor pathway in prostate cancer (Tamboli *et al*., 1998).

Materials and methods

Histology and immunohistochemistry

Mouse prostates from both PB-cdc37 transgenic and normal animals were excised, fixed in 4% formaldehyde in PBS overnight at 4°C, and embedded in paraffin. Sections (5 μm) were made and stained with hematoxylin and eosin (H&E) for histological evaluation. Human prostate specimens including 31 adenocarcinomas, eight benign prostatic hyperplasias (BPH), eight intraepithelial neoplasia (PIN, high grade, $n=6$, low grade, $n=2$) as well as 15 histologically normal prostates from cystoprostatectomies or from organ donors were obtained from the SPORE tissue bank, Baylor College of Medicine, Houston. The specimens were fixed in 10% formalin, paraffin-embedded, and cut into 6 μm sections. Pathological diagnoses, histological evaluation and cancer Gleason score were made on H&E-stained sections by a single pathologist (TM Wheeler).

Cdc37 immunostaining in fixed tissues were performed with rabbit polyclonal affinity purified Cdc37 antibodies as described previously (Stepanova *et al*., 1996). Cdc37 expression in mouse tissues was visualized using FITC-conjugated secondary antibodies. For human prostate specimens, Cdc37 immunostaining was done with the same antibody in conjunction with avidin-biotin-peroxidase (ABC) kit (Vector Lab, CA, USA). Immunoreaction products were visualized using a 3,3′-diaminobenzidine/H_2O_2 solution. Cdc37 immunoreactivity in human prostate specimens was scored at 400× using a Zeiss microscope by counting Cdc37-positive cells. The Cdc37 staining scores ranged from 0: (negative staining); 1+: 0–25%; 2+: 26–50%; 3+: >51–75% to 4+: >75% of cancer cells labeled by the Cdc37 antibody.

Statistical analysis

The correlation of Cdc37 immunoreactivity with cancer Gleason score was determined by Kruskal–Wallis test. Comparisons in Cdc37 staining scores between cancer and normal prostatic or PBH epithelia were made using the Mann–Whitney test. A P value less than 0.05 was considered statistically significant.

Generation of transgenic mice

A PB-Cdc37 transgene was generated by cloning a *Xho*I fragment containing 1.6 kb of the mouse Cdc37 open reading frame (ORF) into a plasmid containing the PB promoter and SV40 splice and polyadenylation sequences. The promoter-transgene cassette was released from vector by digesting with *BssH*II and purified. Transgene DNA was microinjected into male pronuclei of B6D2F1 mouse embryos in the Baylor College of Medicine transgenic core facility. Resulting pups were screened by Southern analysis of genomic DNA isolated from mice tails and digested with *Bam*HI. To establish lines of transgenic mice, founders were continuously mated with ICR mice. All male progeny in the line PB-Cd37.1 were transgenic, while females were not, indicating possible transgene integration into the Y chromosome. Due to the absence of non-transgenic male littermates in PB-Cdc37.1 line, non transgenic littermates of heterozygous PB-Cdc37.2 parents were used as controls for both lines. Phenotypic and histological analyses were performed as described above.

Immunoblotting

Proteins from the indicated samples were extracted and 80 μg of total proteins subjected to electrophoresis on 10% SDS-polyacrylamide gels. Proteins were transferred to nitrocellulose and blotted with affinity purified anti-Cdc37 antibodies. Detection was accomplished using avidin-biotin peroxidase complex procedure. For cancer tissue, samples were selected such that cancerous tissue made up at least 70% of the tissue sample.

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