Journal of Cellular Biochemistry 91:662–670 (2004)

Emergence of Metastatic Hormone-Refractory Disease in Prostate Cancer after Anti-Androgen Therapy

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Abstract The anti-androgens used in prostate cancer therapy have been designed to interfere with the normal androgen receptor (AR)-mediated processes that ensure prostate cell survival, triggering tumor cells to undergo programmed cell death. While anti-androgens were originally designed to treat advanced disease, they have recently been used to debulk organ-confined prostate tumors, to improve positive margins prior to surgery, and for chemoprevention in patients at high risk for prostate cancer. However, tumors treated with anti-androgens frequently become hormone refractory and acquire a more aggressive phenotype. Progression toward metastatic hormone-refractory disease has often been regarded as the outgrowth of a small number of hormone-independent cells that emerge from a hormone-dependent tumor during anti-androgen treatment by natural selection. While a number of selective advantages have recently been identified, there is also considerable evidence suggesting that the progression toward metastatic hormone-refractory disease is an dynamic process which involves abrogation of programmed cell death as a result of the attenuation of DNA fragmentation and maintenance of mitochondrial membrane potential in tumor cells; the upregulation of stromal-mediated growth factor signaling pathways; and the upregulation of extracellular matrix (ECM) protease expression. J. Cell. Biochem. 91: 662–670, 2004.

Key words: invasion; apoptosis; stroma; growth factors

ANTI-ANDROGENS IN PROSTATE CANCER THERAPY

Anti-androgens such as Casodex have been designed to trigger androgen-dependent tumor cells to undergo programmed cell death [Furr, 1996; Furr and Tucker, 1996]. Casodex is now being used as a monotherapy due to its preservation of testosterone levels and sexual potency and has provided an attractive alternative therapeutic approach to surgical intervention [Kolvenbag and Nash, 1999]. However, tumors treated with anti-androgens frequently become hormone refractory and have an increase propensity for metastasis [Grossmann et al., 2001; Kish et al., 2001; Knox and Moore, 2001; Rubben et al., 2001]. Resistant to anti-androgen therapy has been attributed to the intrinsic ability of tumor cells to abrogate the cell death process induced by the anti-androgens. In particular, the progression toward hormone-refractory disease has been related to androgen receptor (AR) status as evidenced by the strong correlation between AR expression and metastatic progression of prostate cancer both in vitro and in vivo [Tilley et al., 1990]. It has also been suggested that failure of conventional androgen deprivation therapy in prostate cancer is caused by the clonal expansion of tumor cells that continue androgen-dependent growth despite of low concentrations of serum androgens through the amplification or mutation of the AR gene [Koivisto et al., 1997]. However, both normal and mutated AR gene expression and amplification have been shown in androgen-insensitive tumors [Wallen et al., 1999; Haapala et al., 2001]. In addition, while Casodex down-regulates nuclear AR level in prostate cancer cells through translocation to the cytoplasm and proteasomal-mediated degradation, transcription of the AR gene does not appears to be altered significantly [Waller et al., 2000; Lee et al., 2003]. Thus, neither mutation

Grant sponsor: Coleman Foundation; Grant sponsor: Department of Defense (an award); Grant number: DAMD-17-02-1-0114-P1.

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Received 12 January 2004; Accepted 13 January 2004

DOI 10.1002/jcb.20040

nor changes in expression of AR can fully account for the loss of androgen-responsiveness and the increased malignancy of prostate cancer cells. Overexpression of *Bcl-2* gene has also been related to the metastatic progression of various cancers, including hormone refractory prostate tumors [Colombel et al., 1993]. However, overexpression of Bcl-2 fails to attenuate Casodex-induced programmed cell death in prostate cancer cells [Lee et al., 2003], suggesting that the increased Bcl-2 expression seen in advance stage prostate tumors is not necessarily the cause for the survival of hormone-refractory cells after anti-androgen therapy. Thus, it is likely that alterations in AR and Bcl-2 expression result from mutations in cancer cells after the induction of hormone-independent tumor and do not initiate the progression to androgen independent disease.

MORPHOLOGICAL CONSIDERATIONS: THE RODENT PROSTATE

The rodent prostate gland is a complex arborized network of ducts, with a number of cell types which display anatomical and biochemical heterogeneity, and substantially different sensitivities to androgen ablation [Cunha et al., 1987]. The secretory epithelial cells are localized in the distal and intermediate regions of the ducts [Sugimura et al., 1986; Rouleau et al., 1990; Sensibar et al., 1991] and are critically dependent on androgens for survival [English et al., 1987]. In the proximal region of the ducts, the luminal epithelial cells display little if any secretory activity [Lee et al., 1990; Rouleau et al., 1990]. Neither these cells, nor the basal cells that are also localized to the proximal region, appear to require androgens for survival. The homeostasis of the prostatic gland is governed by the close reciprocal interaction between the stromal and epithelial tissue compartment [Chung and Cunha, 1983; Cunha et al., 1983; Chung et al., 1984; Miller et al., 1985]. Regression of the rodent prostate is initiated approximately 12 h after castration, when the level of 5α-dihydrotestosterone falls below that needed to sustain survival [Isaacs, 1984]. The reduction in prostate size that occurs over the next 3–6 days is primarily due to the selective loss of the secretory luminal epithelial cells in the distal and intermediate regions, resulting in the complete obliteration of many of the ducts while maintaining the proximal segments of the ducts following castration [English et al., 1987]. The loss of the secretory epithelium without the concomitant loss of stroma results in an increase in stromal–epithelial ratio [DeKlerk and Coffey, 1978]. The reduction of prostatic tumor size after treatment with anti-androgen is primarily due to the selective loss of androgen-dependent tumor cells, resulting in the substantial increase in the stroma–epithelium ratio [Hellstrom et al., 1997]. Since the stromal compartment of the tumor does not undergo significant apoptosis after hormone ablation, this results in a substantial increase in the stromal–epithelial ratio (Fig. 1). Thus, as the tumor cells die, the relative levels of the growth and survival factors secreted by the stroma increases substantially within the tumor.

MORPHOLOGICAL CONSIDERATIONS: HETEROTYPIC INTERACTIONS IN PROSTATE TUMORS

The survival of tumor cells is also dependent on the interaction with the prostate stroma and ECM through intergrin and growth factor-receptor-mediated systems [Cunha et al., 2002; Sung and Chung, 2002]. The interaction between these growth factors including, but not limited to, insulin-like growth factor (IGF), epidermal growth factor (EGF), and members of the fibroblast growth factor families (FGF) such as FGF2, FGF7 and FGF10 and their cognate receptors [Mori et al., 1990; Cohen et al., 1991; Chung et al., 1992; Comoglio and Trusolino, 2002] requires both the expression of the receptor in the tumor cells and the expression and secretion of the ligands from the stroma as well as defined components of the ECM, including chondroitin sulfate and heparan sulfate [Kan et al., 1993]. While their cognate receptors are expressed in the luminal or basal epithelial cells, growth factors such as IGF [Cohen et al., 1991], EGF [Cohen et al., 1994; Freeman et al., 1998], and FGF [Story, 1995; Story et al., 1989; Sherwood et al., 1992; Alarid et al., 1994] are expressed and secreted primarily by the stromal compartment of the prostate. An increase in stroma–epithelium ratio in the prostate tissue thus leads to an increase in local concentration of these growth factors in the tumor cells, activating signaling pathways essential for both proliferation and survival of tumor cells. Increased localization of growth factors such as IGF [Chan et al., 1998a,b; Mantzoros et al.,

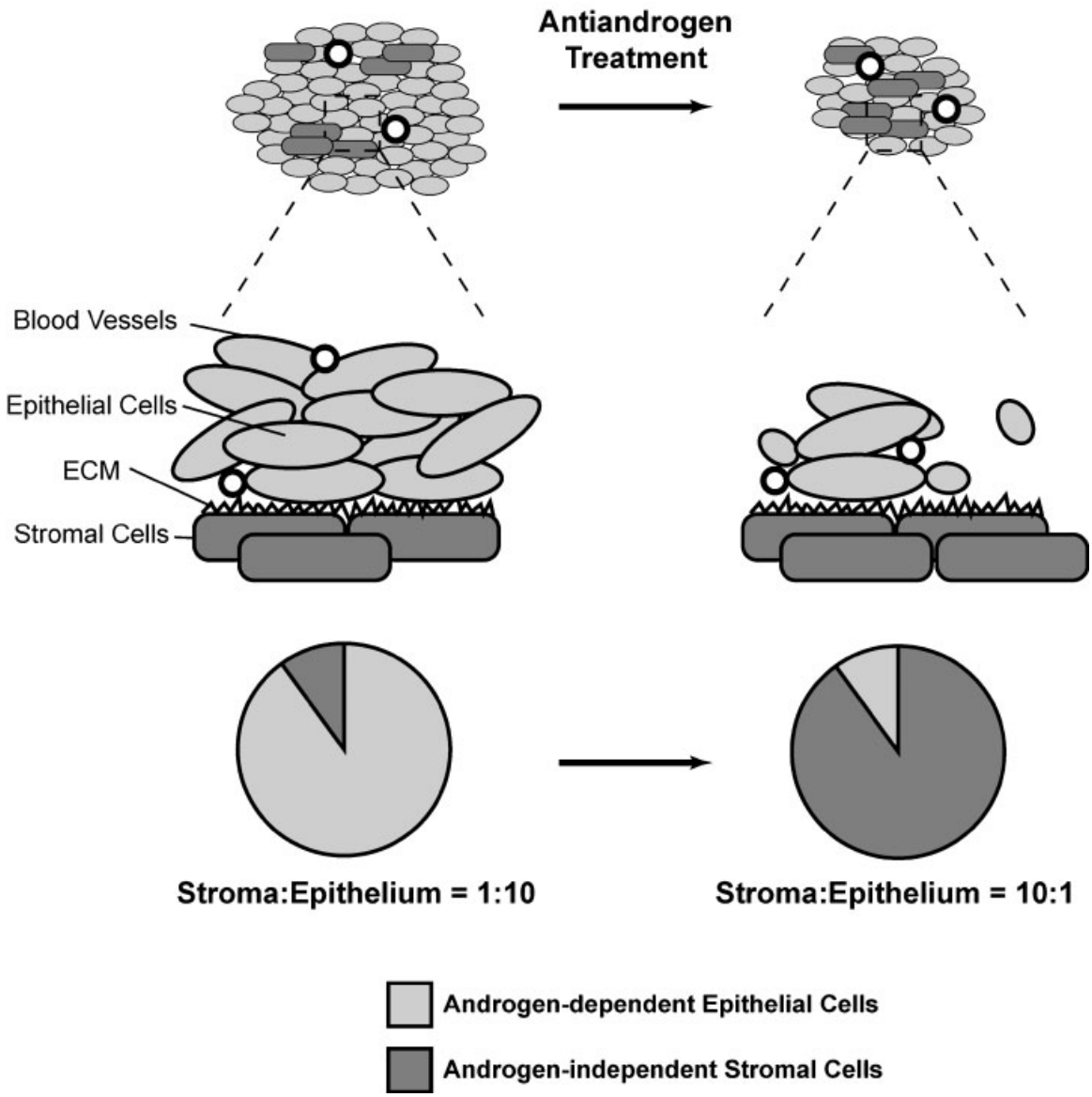

Fig. 1. Changes in stromal–epithelial ratio of prostate tumor after anti-androgen therapy. The reduction of prostate tumor size after anti-androgen therapy is primarily due to the selective loss of androgen-dependent tumor cells, which undergo apoptosis. Since the stromal compartment of the prostate tumor does not require androgens for survival, this results in a substantial increase in the stromal–epithelial ratio after treatment with anti-androgens.

1997; Wolk et al., 1998], EGF [Connolly and Rose, 1990; Hofer et al., 1991; Carruba et al., 1994; McEleny et al., 2002], and FGF [Nakamoto et al., 1992; Cronauer et al., 1997; Dorkin et al., 1999] in the prostate tumor epithelium has been correlated with the metastatic progression of prostate cancer [Sciavolino and Abate-Shen, 1998]. Changes in the relative levels of these growth factors or glycosaminoglycans can influence the induction of apoptosis in glandular epithelial cells and contribute to the cellular micro-heterogeneity during tumor progression [Sugimura et al., 1986; Cunha et al., 1987]. This dynamic interaction between the tumor cells, stroma and ECM is required and responsible for the activation of ras- and PI3-K-dependent signal transduction pathways that appear to be essential for tumor cell survival [McKeehan, 1991; Yan et al., 1992].

Hence, the activation of proliferation and survival pathway by stromal-derived growth factors may override apoptotic-signaling pathways

and render the prostate tumor cells resistant to apoptosis. Moreover, activation of some of these pathways may also promote tumor cell motility, angiogenesis and metastases, suggesting that while anti-androgens may selectively induce cell death of the tumor cells, the resultant increase in the stromal–epithelial ratio may ultimately lead to tumor cell survival and metastatic progression.

FEATURES OF CASODEX-INDUCED CELL DEATH

Mitochondrial Disruption

In contrast to the normal sequence of apoptosis of prostatic epithelial cells after androgen ablation, Casodex induces the loss of cell adhesion prior to the loss of mitochondrial activity [Lee et al., 2003]. Casodex also induces the release of cytochrome *c* without disrupting mitochondria membrane potential ($\Delta\Psi_m$), agreeing with the recent suggestions that the release of cytochrome *c* and disruption of $\Delta\Psi_m$ are separate events in the processes leading to mitochondrial disruption [Achenbach et al., 2000; Loeffler and Kroemer, 2000; Grubb et al., 2001]. Since the release of cytochrome *c* alone does not necessarily lead to cell death in prostate cancer cells [Carson et al., 2002], this suggests that a small but significant portion of Casodex-treated prostate cancer cells can abrogate the death process and remain viable after the loss of cell adhesion.

DNA Fragmentation

Another important feature of Casodex-induced programmed cell death in prostate cancer cells is the lack of low molecular weight (LMW) DNA fragmentation or DNA laddering [Zhan et al., 2002]. Although often regarded as one of the major hallmarks in programmed cell death that occurs during nuclear condensation, DNA fragmentation (especially DNA laddering) is not seen in all cells types undergoing apoptosis, and is clearly not necessary for apoptotic cell death. While most prostate cancer cells appear to have the enzymatic apparatus necessary to complete DNA fragmentation [Marcelli et al., 2000], subtle differences in intranuclear pH, activating divalent cation or inhibiting monovalent cation concentrations may attenuate LMW DNA fragmentation in programmed cell death [Barry and Eastman, 1992]. Regardless of the mechanism of the abrogation of LMW DNA fragmentation, Casodex appears to induce delayed DNA fragmentation which may provide a selection advantage for the emergence of hormone-refractory disease.

Expression of Extracellular Matrix Proteases

During programmed cell death, expression of extracellular matrix (ECM) proteases is induced. The degradation of the ECM is required for the loss of cell-substratum interaction and the apoptotic elimination of superfluous or damaged cells. Casodex also induces a dose-dependent increase in several ECM proteases, including MMP-2 and Cathepsin B during the induction of programmed cell death in prostate cancer cells [Zhan et al., 2002]. Many, if not all, of the ECM proteases that are induced in dying cells are also expressed by metastatic tumor cells and have been associated with the invasive phenotype of these cells [Liotta and Stetler-Stevenson, 1990; Sloane, 1990]. Thus, Casodex-induced up-regulation of pro-invasive ECM proteases during cell death may render the surviving tumor cells that fail to fragment their DNA more invasive and more metastatic.

Stromal-Induced TGFβ Pathway and Metastatic Progression

In normal prostate, the isoforms of transforming growth factor beta (TGFβI–III) are expressed in both stromal and epithelial cells and functions as growth inhibitors [Story et al., 1993, 1996]. Both TGFβ receptors (TGFβ-RI and TGFβ-RII) are abundantly expressed in normal prostate epithelial cells and appeared to be downregulated and exhibit progressive reduction of expression in primary cancer, and lymph node metastases [Guo and Kyprianou, 1999]. In malignant prostate, TGFβ appears to inhibit the immune response and promote angiogenesis, ECM deposition, and metastases [Wilding, 1991; Steiner, 1995]. We have recently shown that increases in stroma volume within prostate tumors lead to a decrease in localization of TGFβ-RI in the tumor cells and an increase in TGFβ-III in the tumor epithelium (unpublished data), suggesting that the increase in stromal–epithelium ratio after treatment with anti-androgens induces a metastatic phenotype by increasing the level of TGFβ, abrogating its inhibitory affect on cell growth by decreasing the level of TGRβ receptors in the prostate tumor epithelium.

Emergence of Hormone-Refractory Metastatic Diseases

Other than mitochondrial disruption and DNA fragmentation, which complete the death process, dying and metastatic cells share an astounding number of similarities. The delayed and incomplete fragmentation induced by Casodex in prostate cancer cells may provide an opportunity for extensive, but inappropriate DNA repair, leading to genomic instability. Because a small portion of non-adherent

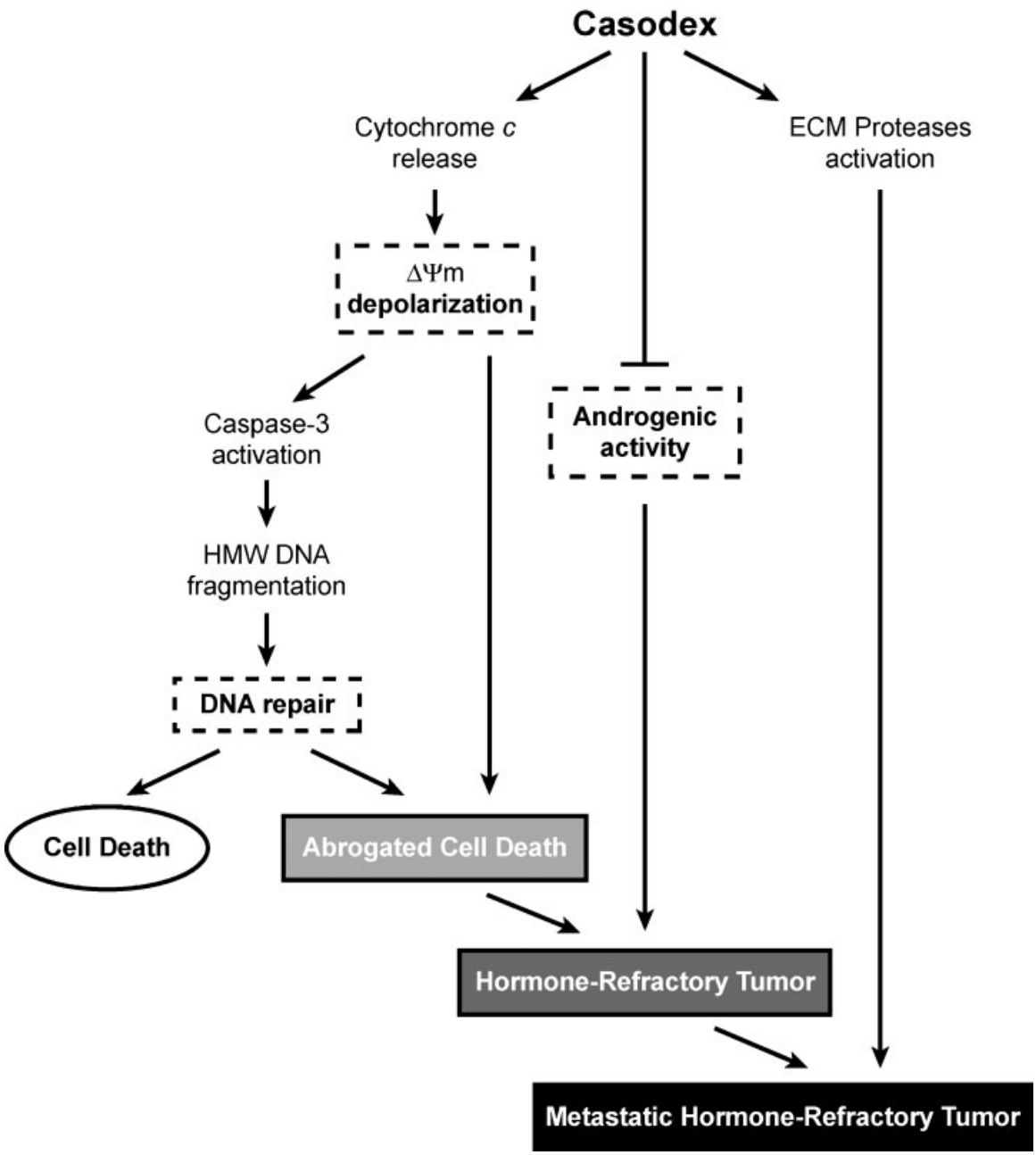

Fig. 2. Intrinsic factors for the emergence of metastatic hormone-refractory disease in prostate cancer after treatment with Casodex. Casodex triggers the programmed cell death processes in prostate tumor cells by interfering with normal androgen receptor (AR)-mediated cell survival process. However, Casodex induces cell death without disrupting $\Delta\Psi_m$ and results in an extended lag phase of cell survival between the initiation of cell death and the fragmentation of DNA. During this time, extensive but inappropriate DNA repair process may occur, producing genomic instability. While only a small portion of these hormone-refractory, genomic unstable tumor cells remains viable, Casodex-induced dose-dependent up-regulation of pro-invasive genes such as ECM proteases also renders them metastatically competent.

prostate cancer cells after Casodex treatment remains viable, Casodex-induced dose-dependent up-regulation of pro-invasive genes such as ECM proteases may also render them also capable of becoming metastatic (Fig. 2).

The microenvironment surrounding the prostate adenocarcinoma cells after treatment with anti-androgens may also plays an important role in inducing an invasive phenotype in these cells that abrogate the apoptotic process. As discussed above, the increase of stromal–epithelium ratio in the prostate tumor after anti-androgen therapy increases the local concentration of a number of growth factors and activates proliferation and survival signaling pathways that override the apoptotic pathways induced by the anti-androgen. Upregulation of stroma-mediated growth factor signaling pathways such as TGFβ may promote cell motility and angiogenesiss and contribute to the induction in metastatic potential. Thus the combination of the intrinsic factors from anti-androgen-induced cell death and extrinsic factors from the microenvironment surrounding these prostate adencarcinoma cells contributes to the emergency of hormone-refractory metastatic diseases.

This is in contrast to the conventional view that resistance to anti-androgens is simply an outgrowth of a small number of hormone-independent cells that emerge from a hormone-dependent tumor during anti-androgen treatment through natural selection. Rather, this hypothesis states that the progression toward

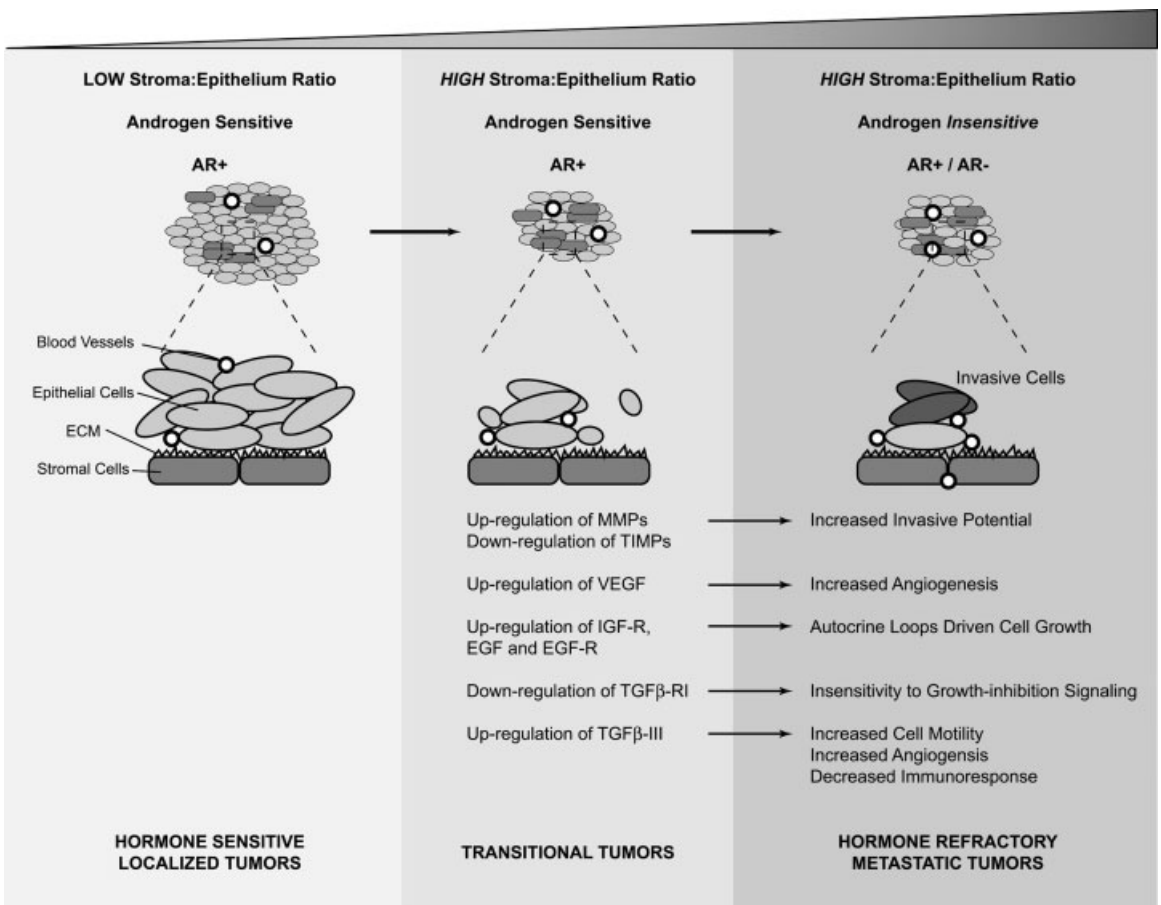

Fig. 3. Schematic diagram of the proposed molecular basis for the emergence of metastatic hormone-refractory disease in prostate cancer after anti-androgen therapy. Anti-androgen reduces prostate tumor size by selectively inducing programmed cell death of AR-positive androgen-sensitive tumor cells and increases the stromal–epithelium ratio within the tumor. The increase in stromal–epithelium ratio leads to the up-regulation of VEGF and TGFβ-III, which enhances the growth of prostate tumors by inducing cell motility and angiogenesis and down-regulation of TGFβ-RI in the tumor epithelium that renders the cells insensitive to growth-inhibition signals. The increase in stromal–epithelium ratio also leads to an upregulation of stromal-mediated growth factor signaling pathways such as the IGF and EGF axis, which further promotes the cell proliferation and survival process and renders the tumor insensitive to androgens.

metastatic hormone-refractory disease is a dynamic process which involves abrogation of programmed cell death as a result of the attenuation of DNA fragmentation and maintenance of mitochondrial membrane potential in tumor cells; the upregulation of stromal-mediated growth factor signaling pathways; and the upregulation of ECM protease expression. This also suggests that the use of anti-androgen in prostate cancer therapy is bound to fail so long as it only targets adenocarcinoma cells in the tumor while ignoring the influence of the microenvironment of the prostate tumor (Fig. 3). This highlights the need to understand the role of the reactive stroma in the maintenance of cell survival after anti-androgen therapy and the need to identify new drug targets to block the dynamic effects of the stroma during anti-androgen therapy.

ACKNOWLEDGMENTS

The authors acknowledge the support of the Marilyn Jane Navari Fellowship in the form of a graduate studentship.

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