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Integrins in prostate cancer progression

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Abstract
Integrins, which are transmembrane receptors for extracellular matrix proteins, play a key role in cell survival, proliferation, migration, gene expression, and activation of growth factor receptors. Their functions and expression are deregulated in several types of cancer, including prostate cancer. In this article, we review the role of integrins in prostate cancer progression and their potential as therapeutic targets.

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Introduction
Prostate cancer develops through a series of defined states: prostatic intraepithelial neoplasia (PIN), high-grade PIN lesions, invasive cancer, and an androgen-independent state (Scher & Heller 2000, Culig & Bartsch 2006, Dehm & Tindall 2006). These defined states arise through multiple alterations in normal cell functions (Mimeault & Batra 2006). Among the alterations described in prostate cancer are abnormal expression and functions of integrins and of their extracellular matrix (ECM) ligands (Boudreau & Bissell 1998, Fornaro et al. 2001). The resulting abnormal cellular interactions with the ECM promote cell proliferation, migration, and differentiation and contribute to cancer progression through the above-described states (Boudreau & Bissell 1998, Fornaro et al. 2001, Knudsen & Miranti 2006). We review here several studies describing aberrations in the normal expression and functions of integrins in prostate cancer, specifically those that are likely to be relevant to the progression of this disease. Due to space constraints, we will not be able to review all the in vitro studies published in this area of research.

Integrin deregulation in prostate cancer
In prostate cancer, tumor cells express an abnormal integrin repertoire and are surrounded by a markedly aberrant ECM. These changes have profound consequences, given the ability of each integrin to regulate specific cell functions. At this time, 24 members of the integrin family, 18 α and 8 β subunits have been described; for a detailed description of the 24 members and for their ECM ligands, the reader should refer to Hynes 2002 and Alam et al. 2007.

Several studies report deregulation of integrin expression as prostate cancer progresses to an advanced stage (Fornaro et al. 2001, Edlund et al. 2004, Knudsen & Miranti 2006; Table 1). Most α and β subunits have been shown to be downregulated in prostate cancer.

Among the α subunits, several reports show that the α3, α4, α5, α7, and αv are downregulated, α1b is upregulated and α2 and α6 are aberrantly expressed as discussed below, whereas there are no reports on the remaining subunits (Table 1). A unique expression pattern has been shown for α2, which is downregulated in prostate cancer but upregulated in lymph node metastases when compared with primary lesions (Bonkhoff et al. 1993). An extensive analysis of α6 expression in prostate cancer has been reported; α6 expression is maintained in prostate neoplasm, but its expression becomes more intense and its density at sites of contact with the basement membrane diminishes with increasing histologic grade (Bonkhoff et al. 1993).

Among the β subunits, β1, β3, and β6 are upregulated in human prostate cancer. β1C and β4 are downregulated, whereas no reports are available for β5, β7, and β8 (Table 1).

Five β1 variant subunits, β1A, β1B, β1C, β1C-2 and β1D, generated by alternative splicing, have been described. Two variants, β1C and β1A, have been shown to be...
Studies performed using cell lines are not included. IHC, immunohistochemistry; TEM, transmission electron microscopy; FACS, fluorescence-activated cell sorting.

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d2 was found to be downregulated in 70% cases with grade II and III tumors.

β1C downregulation was observed regardless of the tumor Gleason grade (grade II to V).

β4 downregulation was observed regardless of the tumor Gleason grade (grade II to V).

Table 1 Integrin deregulation in human prostate cancer

expressed in prostatic epithelium. β1C is shown to be expressed at both protein and mRNA levels in normal prostatic epithelial cells, but is markedly downregulated in adenocarcinoma (Fornaro et al. 1996, 1998, 1999, Perlino et al. 2000). β1A has been found to be consistently upregulated in human prostate cancer (Knox et al. 1994, Murant et al. 1997, Goel et al. 2007) as well as in a mouse model designated TRAMP (transgenic adenocarcinoma of mouse prostate) (Goel et al. 2005). Since β1A associates with many α integrins and α2 and α6 are elevated in prostate cancer, it is conceivable that β1A will be present predominantly as α2β1 and/or α6β1 heterodimeric complexes (Bonkhoff et al. 1993, Fornaro et al. 2001, Alam et al. 2007). The finding that the expression of the β1A integrin variant is upregulated and is necessary for cells to be able to grow in an anchorage-independent manner, point to the important role that β1A integrin may have during prostate cancer progression and will be helpful in formulating new therapeutic strategies (Goel et al. 2005). Recently, increased levels of β1 and its ligand, fibronectin, have been shown to be associated with decreased survival of breast cancer patients (Yao et al. 2007), but this finding has not been reported in prostate cancer.

Upregulation of β3 and β6 integrin variants has been described. Zheng et al. (1999) used human prostate cancer cells isolated from 16 surgical specimens, to show that these cells express α2,β3, whereas normal prostate epithelial cells do not. Similarly, αvβ6 (Azare et al. 2007, Li et al. 2007a) and the truncated αIIbβ6 integrin variant (Trikha et al. 1998a) were found to be expressed in adenocarcinoma.

The β1, β3, and β6 integrin subunits are known to localize in focal contacts and to mediate spreading and cytoskeletal rearrangements in normal cells (Hynes 2002, Alam et al. 2007). However, when we either downregulated or upregulated these subunits by siRNA or ectopic expression analysis, we showed that cancer cell spreading was not affected. These results demonstrate that the ability of β1, β3, and β6 subunits to promote cancer progression is independent of cell spreading.
Expression of $\beta_3$ and $\beta_1$ subunits activates specific signaling pathways and support distinct cancer cell functions. We have discovered that $\beta_3$ is uniquely required in cancer cells for increasing cdc2 levels as well as cdc2 kinase activity. These effects are specific for $\beta_3$ and are not observed for $\beta_6$, although both subunits associate with the same $\alpha$ subunit, $\alpha_v$. Higher levels of cdc2 result in increased cell migration mediated by specific association of cdc2 with cyclin B2 and phosphorylation of caldesmon, a substrate of cdc2. We also demonstrate that cdc2 and caldesmon are localized in the membrane ruffles of motile cells. These results show that cdc2 acts as a downstream effector of the $\alpha_v\beta_3$ integrin and that it promotes cancer cell migration (Manes et al. 2003). In contrast, $\beta_1$ integrin expression did not increase cancer cell motility or cdc2 levels; it appeared, predominantly, to modulate cell proliferation and survival (Manes et al. 2003, Goel et al. 2005). Analysis of the mechanism by which $\beta_1$ may promote tumor growth in vivo shows that $\beta_1$ is uniquely required in cancer cells for localization, expression, and function of a surface receptor — insulin-like growth factor (IGF) type 1 receptor (IGF-IR) which is known to support cancer cell proliferation and survival (Goel et al. 2004, 2005). The mechanism proposed for the control of $\beta_1$ integrin on IGF-IR activity involves the recruitment of specific adaptors to the plasma membrane by $\beta_1$, thus increasing the concentration of specific adaptors proximal to the growth factor receptor (Goel et al. 2004). Evidence is provided in this study that the $\beta_1$ cytodomain plays an important role in mediating $\beta_1$ integrin association with either IRS-1 or Grb2-associated binder1 (Gab1)/SH2-containing protein-tyrosine phosphate 2 (Shp2), downstream effectors of IGF-IR: specifically, $\beta_{1A}$ associates with IRS-1 and $\beta_{1C}$ with Gab1/Shp2 (Goel et al. 2004, 2005). In conclusion, the $\beta_3$ and $\beta_1$ integrins facilitate activation of selective signaling pathways that support cancer progression.

Additional evidence indicates that PTEN, a tumor suppressor frequently deleted or mutated in prostate cancer (Li et al. 1997, Steck et al. 1997), may play a role in the regulation of cell migration on integrin substrates (Tamura et al. 1998, Wu et al. 2007). The mechanism through which it regulates cell migration is not known, although it is likely to utilize PTEN phosphatase activity, which has the ability to dephosphorylate inositol phospholipids such as PIP3 and, as a consequence, to negatively regulate activation of a modulator of motility on integrin substrates, AKT (Alam et al. 2007).

Overall, these findings indicate that the expression of selective integrin subunits is deregulated during prostate cancer progression and that these subunits are potential diagnostic markers in prostate cancer.

**In vivo integrin functions in prostate cancer**

As modulators of cell survival, migration, invasion, and angiogenesis (Parise et al. 2000, Fornaro et al. 2001, Tantivejkul et al. 2004, Moschos et al. 2007) integrins promote progression of many types of cancer including prostate cancer (Fornaro et al. 2001). Preclinical studies (Park et al. 2006, Van Aarsen et al. 2008, Cariati et al. 2008) that have utilized inhibitory antibodies, RGD peptides or siRNA to block integrin functions or expression have shown promising results. We review below the evidence supporting a role for integrins in prostate cancer growth and metastasis in vivo.

**Tumor growth and metastasis**

Primary tumor growth has been shown to be affected by integrin expression. Expression of the $\beta_3$ cytoplasmic variant, $\beta_{3C}$, which is downregulated in prostate cancer, completely prevents tumor growth by inhibiting IGF-IR signaling (Goel et al. 2004). It has also been reported that the expression of $\alpha_7$ in prostate cancer cells injected subcutaneously in SCID mice suppresses tumor growth (Ren et al. 2007). This observation is relevant to the human disease as analysis of $\alpha_7$ in human prostate cancer reveals that $\alpha_7$ is downregulated in this type of cancer and is mutated in 57% of prostate cancers; in addition, mutations in this subunit are associated with increased cancer recurrence (Ren et al. 2007). Pawar et al. (2007) show that growth of tumors treated with fractionated doses of irradiation (3 Gy × 10 days) is inhibited in PC3 cells expressing a mutated non-cleavable form of $\alpha_6$ integrin. Thus, blocking integrin cleavage in vivo may be efficacious for increasing responsiveness to irradiation of pro-metastatic human prostate cancer.

The metastatic process is likely to be dependent on the ability of cancer cells to migrate and invade, but it is also dependent on the ability of these cells to grow in distant sites (Fornaro et al. 2001, Felsing-Habermann 2003). All these functions are mediated by integrins. Early studies using PC3 and DU145 cells, both of which express integrin $\alpha_{4b}\beta_3$, suggest a role for $\alpha_{4b}\beta_3$ in prostate cancer metastasis. These cell lines, implanted subcutaneously or intraprostatically into SCID mice, proved tumorigenic, but only DU145 cells injected intraprostatically metastasized. An analysis performed on the cells described above using an
antibody to α_{IIb}β₃ shows a higher expression of α_{IIb}β₃ in DU145 tumor cells isolated from the prostate when compared with DU145 tumor cells from the subcutis. These data suggest a role for α_{IIb}β₃ in the metastatic progression of prostatic adenocarcinoma (Trikha et al. 1998b). De et al. (2003) use secreted protein acidic and rich in cysteine (SPARC)-deficient mice and show that SPARC selectively supports the migration of highly metastatic as opposed to less metastatic cancer cell lines to bone in an α₃β₃- and α₅β₅-dependent manner.

It should be stressed that ECM proteins are routinely cleaved and their fragments bind integrins (Giannelli et al. 1997, Hynes 2002). The relative ratio of matrix metalloproteinases, cell surface proteases, such as hepsin (Klezovitch et al. 2004) which is upregulated in prostate cancer, and protease inhibitors such as maspin known to be a metastasis suppressor (Li et al. 2004) which is upregulated in prostate cancer bone xenografts (Mahabeleshwar et al. 2006). Finally, a promising avenue is presented by a study showing that the treatment of a PC3 xenograft with an α₅β₃ antagonist (S247, a cyclic RGD peptidomimetic) in combination with radiation, leads to enhanced anti-angiogenic and anti-tumor effects when compared with either therapy alone (Abdollahi et al. 2005).

The use of integrin inhibitors is likely to affect both cancer cell survival and angiogenesis since integrins are expressed by tumor cells as well as by endothelial cells. Although it is hard to discriminate between an effect on tumor growth and an effect on angiogenesis, a maximal response of these inhibitors can be predicted when the targeted integrin is expressed by both tumor and endothelial cells.

These preclinical studies which take advantage of the available mechanistic investigations, have prompted several clinical studies (described below), aimed at identifying novel molecular strategies to block prostate cancer progression.

Integrin inhibitors in clinical trials

Clinical trials that evaluate the effect of integrin antagonists as prostate cancer therapeutics are

Tumor angiogenesis

Angiogenesis, a process critical for tumor formation and growth (Nicholson & Theodorescu 2004, Jimenez et al. 2006, Sakamoto et al. 2008), is regulated by integrin functions (Hynes 2007). α₅β₃ and α₅β₅, by using a crosstalk with growth factor signaling pathways (Alam et al. 2007), regulate angiogenesis. Sun et al. (2007) have evaluated the efficacy of a new camptothecin conjugate, JF-10-81, an anti-angiogenic agent, in a prostate cancer mouse model. JF-10-81 blocks cancer cell adhesion in vivo and angiogenesis in C57Bl/6 mice and reduces expression of α₅β₃ and α₅β₅ on PC3 cells which implies an inhibitory effect on angiogenesis through an α₅β₃- and α₅β₅-dependent mechanism. A study that analyzes a knock-in mouse expressing a mutant β₃ that cannot undergo tyrosine phosphorylation shows that β₃-deficient mice have impaired capillary formation in response to VEGF stimulation, and thus form smaller prostate tumors than their wild-type counterparts. These observations highlight the role of vascular α₅β₃ in prostate cancer through modulation of angiogenesis (Mahabeleshwar et al. 2006). Finally, a promising avenue is presented by a study showing that the treatment of a PC3 xenograft with an α₅β₃ antagonist (S247, a cyclic RGD peptidomimetic) in combination with radiation, leads to enhanced anti-angiogenic and anti-tumor effects when compared with either therapy alone (Abdollahi et al. 2005).

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ongoing. Available reports at this time indicate that the αv integrins are promising therapeutic targets in prostate cancer. Two clinical trials using Cilengitide, a cyclic Arg-Gly-Asp peptide that inhibits αvβ3 and αvβ5 (Beekman et al. 2006), an antagonist of αv integrins, are in progress. Cilengitide is being evaluated in two Phase II clinical trials. In one study (NCI 6735), one dose of 2000 mg given intravenously twice weekly is being evaluated in men with androgen-independent prostate cancer and non-metastatic disease. In another study, (NCI 6372), two dose levels of Cilengitide, 500 and 2000 mg, are administered twice weekly in men with androgen-independent metastatic prostate cancer (Beekman et al. 2006). Antibodies to αv integrins are also being evaluated in two clinical trials. The first utilizes CNTO 95, a monoclonal antibody that inhibits αv integrins and blocks tumor growth (Chen et al. 2007). In Phase I, CNTO 95 (10 mg/kg, once a week) in combination with standard drugs docetaxel (75 mg/m², every 3 weeks) and prednisone (twice a day) appears to be well tolerated in hormone refractory prostate cancer patients (Chu et al. 2007). A Phase II clinical trial (NCT00537381) is also in progress with CNTO 95 (10 mg/kg, once a week) in combination with docetaxel (75 mg/m², every 3 weeks) and prednisone (twice a day) in metastatic hormone refractory prostate cancer patients. The second trial utilizes MEDI-522, a humanized monoclonal IgG1 antibody directed against the αvβ3 integrin. MEDI-522 blocks the binding of ligands, such as vitronectin, to αvβ3 integrin (McNeel et al. 2005). A Phase II, randomized, open-label, two-arm, multicenter study of MEDI-522, in combination with docetaxel, prednisone, and zoledronic acid in the treatment of patients with metastatic androgen-independent prostate cancer (NCT00072930) is ongoing. Results from these studies will pave the way to new and improved strategies to prevent prostate cancer in humans.

Conclusions
Prostate cancer accounts for a significant cancer burden in the USA, where it is projected to result in over 28 660 deaths and more than 186 320 new cases in 2008 (Jemal et al. 2008). While substantial advances have been made in diagnosing and treating this disease, the molecular mechanisms that promote prostate cancer progression remain to be fully investigated (Pomerantz & Kantoff 2007). The studies reviewed here show that prostate cancer progression has been correlated with expression of specific integrin subunits and is influenced by deregulation of selective subunits, which then activate distinct signaling pathways (Fornaro et al. 2001). Although the molecular pathways by which integrins contribute to cancer progression need to be fully elucidated, designing new therapeutic approaches for prostate cancer based on inhibiting integrin expression, Ligand binding or downstream signaling is likely to be a successful strategy.

Several questions remain to be answered in this under-investigated area of research. The mechanisms by which integrins are regulated need to be characterized by focusing on modulators of integrin expression. Yet another promising avenue of research is to elucidate the role of integrins in promoting proliferation of prostate cancer stem cells, in particular, αvβ1 appears to be interesting since it is highly expressed in these cells (Collins et al. 2005, Mimeault et al. 2007). Finally, an under-investigated area in prostate cancer research is the cross talk between bone microenvironment and metastasis (Mohla 2004), to which the interactions between integrins and their ECM ligands are likely to contribute significantly. Since integrins mediate the interactions between tumor cells and bone microenvironment and facilitate growth in bone, a potential application of the use of integrin inhibitors is to prevent prostate cancer bone lesions (Waltregny et al. 2000, Pecheur et al. 2002, Karadag et al. 2004, Hall et al. 2006). These lesions are osteoblastic or osteolytic and are frequently detected in prostate cancer patients (over 80% of prostate cancer patients have established bone metastasis at autopsy (Koeneman et al. 1999)). A recent study has shown that the αvβ3 integrin promotes bone gain mediated by prostate cancer cells that metastatize to the bone and point to αvβ3 as a potential therapeutic target to block prostate cancer osteoblastic lesions (Keller & Brown 2004, McCabe et al. 2007). Besides therapeutic applications, other uses of integrin inhibitors are in the area of imaging and specific delivery of a drug to cancer cells (Chen et al. 2001, Moschos et al. 2007, Li et al. 2008).

In conclusion, these investigations indicate that the clinical use of integrin inhibitors is likely to be a successful strategy to prevent all stages of cancer progression from tumor growth to metastasis.

Declaration of interest
The authors declare that there is no conflict of interest that would prejudice the impartiality of this work.

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