β-Catenin signaling in prostate cancer: an early perspective

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Abstract

Further understanding of the molecular mechanisms responsible for prostate cancer (CaP) development and progression is paramount for overcoming the current diagnostic and therapeutic hurdles presented by this urologic disease. The β-catenin nuclear signaling molecule has been widely implicated as an oncogene in human cancer, including CaP. Pooling together knowledge gathered on the contributions of β-catenin and other factors to human neoplasia may assist in the development of better strategies for management and treatment of prostate tumors of all stages (early, advanced/androgen-dependent, advanced/androgen-independent). Although there is considerable lack of comprehension regarding the function of β-catenin transcriptional activity in prostate tumors in vivo, recent evidence indicates the probability that β-catenin contributes to multiple signaling pathways for which a causative role in CaP is already known. In this review, we will approach such pathway interactions, perhaps the most notable being androgen receptor (AR) signaling, in order to highlight those avenues through which β-catenin may exert its cancer-related function. To the same end, we will draw attention to normal β-catenin signaling in the prostate; however, as only very limited knowledge exists on this topic, much of the discussion will be correlative. Our final topic will concentrate on how, given realistic scenarios of androgen stimulation or absence in both normal and neoplastic prostate cells, nuclear β-catenin may ultimately potentiate wnt cell–cell signaling and AR activities. Heightening our comprehension of β-catenin signaling mechanisms and its phenotypic consequences in CaP – and in normal prostate – may contribute to that body of knowledge which will eventually prove useful for devising more effective therapies.

Introduction

Recent studies have begun the process of understanding the role β-catenin signaling may play in prostate cancer (CaP). In this review, we report on these new findings in order to provide a general framework from which to further study this question. We hope that our discussion will ultimately be of benefit in addressing two fundamental problems confronting CaP researchers: an incomplete comprehension of those physiological mechanisms underlying (1) prostate tumorigenesis and (2) CaP progression. Clinically speaking, understanding both these problems could be beneficial for developing reliable diagnostic markers and novel therapeutic modalities. Regarding the latter, advances would be highly welcomed that yield therapies which more effectively block progression of androgen-dependent (AD) disease to that which is androgen-independent (AI). AD tumors regress with hormonal ablation therapy, but almost always relapse to a more lethal, hormone-refractory state. The β-catenin oncogene product is proposed to be involved in the natural history of several human tumors (Morin 1999, Polakis 1999). Since the androgen receptor (AR) is known to be a major determining factor in processes key to understanding CaP (cell proliferation, differentiation, quiescence), and is itself targeted in hormonal therapy of AD tumors, we will devote some discussion to concentrate on potential forms of AR/β-catenin cross-regulation and its consequences. In addition, we will consider the much better understood role of β-catenin in mediating the signaling of wnt factors; however, at this time we only offer a limited perspective for this pathway in the prostate by broaching β-catenin activities already described for other tissues. We stress that the role of β-catenin in CaP – not to mention that in normal prostate – is still very much unknown. Careful examination of recent reports linking β-catenin to important aspects of this disease should shed
light on established paradigms of prostate tumor progression and possibly invoke new mechanisms.

**Studying β-catenin function**

Recent research has greatly expanded the understanding of β-catenin nuclear signaling (Willert & Nusse 1998, Miller et al. 1999, Hecht & Kemler 2000). By the late 1980s, β-catenin was known to be a structural component of adherens junctional complexes (Ozawa et al. 1989); however, data from the early 1990s connecting the *Drosophila* homologue of β-catenin (armadillo) to wingless cell–cell signaling (Riggleman et al. 1989, 1990, Noordermeer et al. 1992), coupled with the discovery of alternate β-catenin binding partners such as the adenomatous polyposis coli (APC) tumor suppressor (Rubinfeld et al. 1993, Su et al. 1993), provided an early impetus to describe the otherwise unknown role of β-catenin in signal transduction. Much of the work on β-catenin signaling up to the present spawned from these initial studies of development and tumorigenesis. In reference to the latter area of interest, direct activating mutations in β-catenin, as well as inactivating mutations of its regulators such as APC, have recently been discovered in multiple human cancers including CaP (Voeller et al. 1998, Chesire et al. 2000, Gerstein et al. 2002). Despite this surge in interest, inquiries into how nuclear β-catenin activity is regulated and what its outcomes are in normal prostate and CaP have been preliminary in nature. Perhaps this scarcity of knowledge regarding β-catenin activity in prostate derives from two main issues: (1) from a methodological standpoint, human prostate is generally more difficult to study compared with some other tissues (e.g. low availability of cell line models and pathological specimens, particularly those from disseminated disease) and (2) β-catenin mutation analyses have estimated putative activating mutations to be relatively uncommon (~5%). Several tantalizing discoveries that may elucidate facets of β-catenin function in CaP, however, have since sparked renewed interest in this transcription factor (Truica et al. 2000, Chesire et al. 2002, Mulholland et al. 2002, Pawlowski et al. 2002, Yang et al. 2002). These sort of inquiries embody the notion that tissue specificity exists at the molecular level; hence, pathways must be dissected in a tissue-specific manner. We outline here some of the working hypotheses for studying the regulation and physiological consequences of β-catenin signaling.

**The canonical wnt/β-catenin signaling pathway**

Providing a simple outline of the wnt/β-catenin pathway unavoidably belies its actual complexity; therefore, we refer readers to more comprehensive reviews of this topic (Cadigan & Nusse 1997, Moon et al. 2002). β-Catenin was first described as a structural component in adherens junction formation, a mechanism of cell–cell adhesion. The participation of β-catenin therein renders it, along with other cytoskeletal proteins, relatively resistant to non-ionic detergent extraction (i.e. insoluble). Conversely, that β-catenin population which is easily extracted (i.e. soluble) exists in cytoplasm/nuclear complexes and can be viewed as a potential effector molecule of wnt signaling – basically, acting as a relay from the membrane (stimulus input) to the nucleus (transcriptional output). We will be focusing herein on the soluble form of β-catenin, except as otherwise noted.

Wnt factors comprise a large number of secreted glycoproteins that shape morphogenic processes, partly through stimulating target cell β-catenin transcriptional function (Fig. 1) (Cadigan & Nusse 1997, Moon et al. 2002). Cells respond to wnt ligands via expression of the frizzled (fzd) family of receptors and low density lipoprotein receptor-related proteins (LRP) which act as wnt co-receptors – wnt/fzd/LRP complex formation is requisite for prompting β-catenin activity. Noteworthy here is that wnt factors are able to induce alternate signal transduction cascades aside from the ‘canonical wnt/β-catenin signaling pathway’ (Miller et al. 1999) (Fig. 1). This diversity in wnt responses likely derives, in part, from certain molecular phenomena occurring at the cell/extracellular interface (Zorn 2001, Kawano & Kypa 2003).

β-Catenin is normally maintained at low levels in the absence of wnt stimulation due to its constitutive proteasomal degradation (Hart et al. 1999). The regulators that confer this turnover, APC and Axin, position β-catenin for phosphorylation at its N-terminus (encoded by human exon 3) by glycogen synthase kinase-3β (GSK-3β) (Fig. 1). The targeted serine and threonine residues reside in an 1κB consensus destruction motif and upon their phosphorylation by GSK-3β prime β-catenin for ubiquitination and subsequent proteasomal degradation. The latter processes necessitate the factor β-transducin repeat-containing protein (β-TrCP1), which recognizes phosphorylated β-catenin and initiates its ubiquitination. De-repression of this post-translational regulation of β-catenin through inhibition of GSK-3β kinase activity is induced downstream formation of an appropriate wnt/fzd/LRP complex, hence triggering β-catenin accrual. Although the mechanism at play between wnt binding and GSK-3β suppression is not well understood, recent work in this area has been very illuminating (Peters et al. 1999, Liu et al. 2001).

The relaxation of β-catenin downregulation upon wnt stimulation leads to greater β-catenin participation in at least two processes, nuclear signaling and cell–cell adhesion. Although these two paths are outwardly different, they are now proposed to be somewhat inter-twined; the latter may shape the degree of the former. β-Catenin accumulation results in its translocation into the nucleus and interaction with DNA-binding transcription factors, the most prominent of which are those of the TCF/lymphoid enhancer factor (LEF) family. The mechanism(s) responsible for β-catenin
Figure 1 A view of the canonical wnt/\(\beta\)-catenin pathway. The canonical pathway enhances \(\beta\)-catenin stability and consequent nuclear translocation. In the nucleus, it displaces co-repressors from T cell factor (TCF) DNA-binding proteins and trans-activates target gene expression through recruitment of the RNA transcription initiation complex. Shown in the shaded box is a schematic representing a wnt signaling event which does not proceed to activate \(\beta\)-catenin. \(\beta\)-Catenin has been proposed, in prostate cells, to interact with other pathways (see Fig. 4). \(\beta\), \(\beta\)-catenin; Dkk1, Dickkopf; Dsh, Disheveled; ubq, ubiquitin groups/polyubiquitination; CBP, CREB-binding protein; TBP, TATA-binding protein; HDAC, histone deacetylases; CtBP, C-terminal binding protein; \(P_i\), inorganic phosphate; CRT, catenin-regulated transcription.
nuclear import has been shown to occur independently from importin protein interaction and may incorporate heretofore unrecognized processes (Fagotto et al. 1998, Mulholland et al. 2002). Once localized to the nucleus, β-catenin has been proposed to face both nuclear export and retention pressures by APC and TCF, respectively (Henderson 2000, Rosin-Arbesfeld et al. 2000, Henderson et al. 2002).

Functional output of wnt/β-catenin signaling (i.e. transcriptional regulation) commences upon nuclear localization of ‘activated’ β-catenin (Fig. 1). Most studies thus far have built on the model which is based on β-catenin upregulation of target gene expression via its trans-activation of TCF/LEF transcription factors. The four members of this DNA-binding factor family are TCF1, TCF3, TCF4, and LEF1; TCF3 expression is restricted to stages of development, whereas the others are expressed widely, but not universally, in adult tissues (Zhou et al. 1995, Barker et al. 1999, Roose et al. 1999, Hovanes et al. 2001). Most CaP cell lines express TCF4 and LEF1 (authors’ unpublished data), although measurement of TCF expression in prostate tissue has not been reported. In the absence of nuclear β-catenin, TCF binds to specific sequences contained in promoters of target genes and mediates repression of gene expression via its collaboration with certain repressors (Cavallo et al. 1998, Roese et al. 1998, Kim et al. 2000). TCF interaction with β-catenin propagates target gene expression: β-catenin competes with repressors for TCF binding and recruits trans-activating factors to its trans-activation domain(s). This form of β-catenin transcriptional activity has been termed CRT (Morin et al. 1997); hence, we will likewise use this term in this review. To emphasize the notion that this pathway is not completely understood, we point out that there exist contradictory reports regarding the nature of the β-catenin/TCF interaction and its consequent role in target gene induction (Chan & Struhl 2002, Bienz & Clevers 2003).

TCF proteins do not hold a monopoly on nuclear β-catenin; the latter has been shown to modify the activities of other DNA-binding factors including Sox-17-β and certain steroid receptors (Easwaran et al. 1999, Zorn et al. 1999, Truica et al. 2000, Palmer et al. 2001). Several intriguing observations speak to the potential complexity underlying CRT regulation: expression of multiple TCF splice forms (van de Wetering et al. 1996, Brannon et al. 1999, Duval et al. 2000), TCF/LEF modification by phosphorylation and SUMOylation (Ishitani et al. 1999, Sachdev et al. 2001), participation of TCF in transcription potentially unrelated to that induced by wnt signaling (Bruhn et al. 1997, Hsu et al. 1998, Labbe et al. 2000, Nishita et al. 2000), and that several co-factors are known to modify β-catenin-mediated transactivation of TCF (Eastman & Grosschedl 1999, Hecht & Kemler 2000). Interestingly, this long list may only scratch the surface of potential CRT control mechanisms, as all these points may be subject to modification as a function of tissue-specific, temporal, and spatial inputs. Our discussion on β-catenin’s role in prostate will include recent findings showing that β-catenin augments AR transcriptional activity and, conversely, that AR activity may attenuate CRT. Here, the latter example of this mutual cross-regulation may exemplify a degree of tissue-specific CRT regulation.

Although β-catenin is now proposed to directly modify the transcriptional output of DNA-binding factors besides TCF, much emphasis has been placed on identifying CRT – again, β-catenin/TCF-mediated transcription – target genes. A comprehensive list of target genes can be found at the Wnt Gene Homepage (www.stanford.edu/%7Ermusse/wntwindow.html). These and as-of-yet undiscovered target genes are likely subject to multiple tiers of regulation, thus making the possibility of multi-specific target gene expression very high. This proposition may aid in explaining the enigmatic observation that certain CRT target genes, such as cyclin D1, are not accordingly induced in a universal fashion across a series of different tissues (Tetsu & McCormick 1999, Cadoret et al. 2001, Michaelson & Leder 2001, Gounari et al. 2002, van de Wetering et al. 2002).

Morphogenic aspects of β-catenin signaling

CRT-induced phenotypes have been inferred, in part, from assessing target gene product function; however, very little is known regarding how certain sets of induced genes actually orchestrate a particular outcome. In mice and humans, clues towards determining these complex phenotypic effects have been garnered through investigation of both normal and pathologic states in which β-catenin activity is proposed to arise. The status of β-catenin in a tissue of interest can be revealed through immunohistological analysis – nuclear staining is a hallmark of β-catenin activation and may be useful in correlating its transcriptional function to certain aspects of tissue homeostasis (van de Wetering et al. 2002). Extending this modus operandi are staining studies examining the expression of known CRT targets (Batlle et al. 2002, Leung et al. 2002), as well as transgenic models that allow one to assess CRT on a global level through monitoring expression of a proxy target gene (DasGupta & Fuchs 1999, Dorsky et al. 2002). The normal states in which β-catenin signaling critically functions in mammals are numerous, including embryonic anterior–posterior axis specification (Huelsken et al. 2000) and tissue development. With respect to the latter area of interest, one goal is to determine if any β-catenin-dependent developmental phenotypes mirror those manifested in neoplasia – certain facets of cancer are reminiscent of those underlying embryogenesis.

Several groups have undertaken the study of nuclear β-catenin/CRT function in tissue morphogenesis using transgenic mouse models, the results of which may elucidate such a role for β-catenin in prostate formation. Both the high frequency of APC/β-catenin pathway alteration in colon cancer (Kinzler et al. 1991, Sparks et al. 1998, Miyaki et al.)
Probing β-catenin signaling in the prostate in vivo

Through the use of transgenic mice, Gounari et al. (2002) have provided a glimpse of the potential effects of β-catenin signaling on prostate physiology. Their strategy employed mice harboring a β-catenin allele in which the regulatory domain (see next section and Fig. 2a) is deleted in cells expressing Cre recombinase from the mouse mammary tumor virus-long terminal repeat (MMTV-LTR) regulatory element (promoter); otherwise, the targeted locus remains intact, utilizing its natural transcribed and non-transcribed regulatory sequences. The MMTV-LTR element is responsive to androgen; hence, all four prostatic lobes of experimental male transgenic animals (Catnb+/-lox(ex3)) were shown to express a constitutively active form of β-catenin (i.e. regulatory domain-deleted β-catenin). Importantly, Gounari and colleagues report that, in these mice, formation of prostatic intraepithelial neoplasia (PIN)-like lesions occurs concomitantly with β-catenin stabilization. This phenotype was correlated with ectopic nuclear β-catenin staining and upregulated expression of c-myc, a CRT target gene. PIN, a proliferative lesion (dysplasia) believed to precede development of prostatic carcinoma, consists of cells bearing secretory-like morphology and gene expression (Montironi et al. 2002). To what extent does this particular outcome of cellular expansion coupled with differentiation faithfully reflect the singular effects of dysregulated β-catenin signaling in prostate? Or taken another way, does this mouse adequately model the selected role – possibly proliferation – of β-catenin mutation in a subset of CaPs (see next section)? Studies are warranted to compare this new genetic model with others for which a prostate phenotype may be predicted to exist a priori.

Although still conjectural, secreted wnt factors are proposed to exert their growth effects on stem cells of individual tissues (Korinek et al. 1998, Reya et al. 2003): hence, by correlation, we are intrigued by the possibility that a major aspect of β-catenin signaling in normal prostate physiology is exacted through such precursor cells. These ‘elusive’ cells are purported to reside in the basal compartment of gland acini in humans and mice (Isaacs & Coffey 1989, De Marzo et al. 1998b, Wang et al. 2001, Tsujimura et al. 2002) and are predicted to be an absolute requirement for not only normal processes of fetal and adult prostate organogenesis, but also for testosterone-induced regeneration of glands involuted as a consequence of perturbing androgen stimulation (Montpetit et al. 1998, De Marzo et al. 1998b). In brief regards to the latter point, such cycling studies generally entail animal castration which promptly spurs atrophy and apoptosis of luminal cells, thus shrinking the gland and leaving predominantly basal epithelia. This step is followed by administration of testosterone which induces appropriate gland re-growth (Kerr & Searle 1973, Sandford et al. 1984) (see Fig. 6a). If β-catenin signaling comprises a major part of the downstream signaling events triggered in prostate cells by wnt factors, and if wnt signaling does shape prostate morphogenesis, then it may be fair to refer to the transgenic mice described by Gounari et al. (2002) to address this matter.

That a subset of prostate basal cells are androgen responsive (i.e. AR-positive and capable of MMTV-LTR-driven transcription) suggests that the altered prostate phenotype manifested by Catnb+/-lox(ex3) animals may result from elevated stem cell expansion and/or maturation, thereby imitating, albeit grossly, the putative effects of wnt stimulation. This conjecture may be supported by the observation in Catnb+/-lox(ex3) mice of small patches of abnormally expanded basal cells, perhaps a consequence of their accrual of nuclear β-catenin. However, as the authors note, it is difficult to determine if the predominant Catnb+/-lox(ex3) phenotype (luminal cell dysplasia) results from ectopic β-catenin signaling in the basal compartment, potentially spurring progenitor cell proliferation and differentiation, or specifically from β-catenin signaling in the secretory compartment.
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Figure 2  (a) The regulatory domain of β-catenin which is targeted for constitutive serine/threonine phosphorylation by GSK-3β. Phosphorylation sites are indicated by arrow heads. The curved, broken arrows indicate the order in which serine/threonine substrates are recognized by the GSK-3β enzyme (Cohen & Frame 2001). Recent work by Liu et al. (2002) suggests that serine 45 is targeted specifically by casein kinase Iα. Three independent investigations for β-catenin alteration in CaP are shown and listed below each is the number of changes detected per number of cases (localized CaP mutation rate, advanced CaP mutation rate). Certain xenografts were utilized in two of these studies. Amino acid changes by missense mutation and deletion are appropriately noted and are heterozygous. Except as noted for the Δ24–47 mutation, all genotypes were found in primary tumors. Δ, deleted codon(s); *, mutation occurring in a xenograft (PC82) which was derived from primary cancer; †, this interstitial deletion was observed in all metastases obtained from a hormonal failure case (i.e. clonal). (b) Immunohistological analyses show β-catenin expression in CaP specimens. β-Catenin (brown) is counterstained with the DNA-staining dye hematoxylin. Atop each panel is the genotype for the GSK-3β-phosphorylation domain of β-catenin. The first three panels show specimens of localized, AD disease, whereas the fourth panel shows a metastatic, AI specimen. Note that in the first panel (wildtype (WT)), β-catenin staining is predominantly at cell–cell borders despite the rather anaplastic morphology of the tumor. This result contrasts with those tumors bearing mutant β-catenin alleles (second, third and fourth panels) which contain elevated cytoplasmic and nuclear β-catenin, the latter of which is heterogeneous within each tumor. S, serine; P, proline; D, aspartate; G, glycine.

Interrogation of β-catenin signaling function in the prostate need not only incorporate studies based on transgenic modulation. Simple histological analyses have associated nuclear β-catenin staining with certain facets of tissue development and therefore would be amenable to making similar inquiries in the prostate (Ridanpaa et al. 2001, Tebar et al. 2001, Everett et al. 2002, van de Wetering et al. 2002). Eberhart & Argani (2001) demonstrated this point through a survey of a wide range of human tissues including those at various embryonic stages of development; although prostatic secretary cells were found to exhibit heterogeneous nuclear positivity, this result is of limited value as it was obtained from the study’s only prostate specimen (1.5 years of age). More extensive analyses of embryonic and prepubertal
human prostate tissue are called for and could yield very useful insights. In related work, we and colleagues failed to detect nuclear β-catenin in prostatic buds of embryonic day 19.5 male mice (D Berman, unpublished data); however, this result does not preclude the possibility of nuclear localization occurring during the earlier phases of epithelial bud invasion into the urogenital sinus (buds emerge at embryonic day 17) or immediately after embryonic day 19.5 when ductal branching begins.

Evidence for β-catenin pathway involvement in CaP

The large emphasis placed on solving the molecular bases of cancer usually pays out twofold dividends: both the basic physiology of a tumor and its normal tissue counterpart are uncovered. Discovery of the int-1 oncogene (otherwise known as wnt-1) by Nusse & Varmus (1982) as a factor in murine breast carcinoma initiated the study of wnt signaling in cancer. Only limited expression analyses have been embarked upon to examine wnt factors and their receptors in the prostate (Iozzo et al. 1995, Katoh et al. 1996, Sagara et al. 1998, Katoh 2001, Kirikoshi et al. 2001, Saito & Katoh 2001). Despite the fact that certain wnt factors upregulate β-catenin activity, they themselves have not been implicated largely in human neoplasia. Could this apparent anomaly be related to the wide pleotropic effects of wnt signals (see Imbert et al. 2001 and references therein)? If so, perhaps cell selection is elicited only upon direct activation of β-catenin. As previously discussed, β-catenin activity is associated with proliferation and differentiation, implying that the context (i.e. timing within a multi-hit model for tumor progression) in which β-catenin upregulation occurs is important to consider.

Downstream components of the canonical wnt/β-catenin-signaling pathway have been implicated in several human cancers including that of prostate. Currently, the pathway component most conspicuously associated with CaP is β-catenin itself (Voeller et al. 1998, Chesire et al. 2000, 2002, Gerstein et al. 2002, de la Taille et al. 2003). Aberrant upregulation of β-catenin nuclear activity, and therefore CRT, can result from direct mutation of β-catenin’s regulatory domain, ultimately impinging on its phosphorylation by GSK-3β. Although the mutations generally involve certain GSK-3β-targeted serine and threonine residues, alterations affecting residue 32 (aspartate) also occur. Such activating mutations in CaP are predominantly found to occur at a low rate (~5%) in primary lesions (i.e. AD disease) and consist mainly of missense changes (Fig. 2a). Maybe of little surprise, but nonetheless important to know, these mutations impart greater stability to β-catenin in prostate cells as measured in vitro (CRT assays) and in vivo (immunohistological detection of nuclear localization, Fig. 2b) (Cheshire et al. 2000, Gerstein et al. 2002). These data culminate with the idea that mutant β-catenin in CaP does display a definitively altered metabolism consistent with that documented in other human cancers (Morin 1999, Polakis 1999), and that such a phenotype is selected under fairly limited/narrow conditions.

The APC and β-TrCP1 components of the β-catenin destruction complex (Fig. 1) have been linked to CaP etiology. One study showed loss of heterozygosity at the APC gene locus, but these data have not been reproduced elsewhere (Phillips et al. 1994). More recently, Gerstein et al. (2002) demonstrated that ~16% (3/19) of CaP specimens (xenografts and frozen tissue) bear deleted and truncation-encoding APC alleles. This tumor suppressor is similarly nullified in other cancers, especially that of the colon (Kinzler & Vogelstein 1996, Bienz 2002) – these changes render β-catenin resistant to GSK-3β phosphorylation and subsequent proteasomal degradation. In addition to gene loss, abrogated expression of APC in progressive CaP may be related to promoter hypermethylation (Maruyama et al. 2002), although more work is necessary to fully determine the allelic nature of this epigenetic phenomenon. Earlier investigations for APC mutation in Japanese CaP cases were negative (Suzuki et al. 1994, Watanabe et al. 1996); however, this overall result may reflect different selective processes existing between eastern and western ethnicities, as disparity is likewise manifest with studies of ras mutation frequency (Carter et al. 1990, Konishi et al. 1992, Moul et al. 1992, Suzuki et al. 1994). Offering another point of view, Gerstein et al. (2002) postulated that truncating APC mutations may not have been readily detected in earlier studies for technical reasons. More genetic and functional analyses could provide a greater appreciation for APC involvement in CaP.

Discovery that Axin-1 and -2 may behave as tumor suppressors in certain malignancies (Liu et al. 2000, Satoh et al. 2000, Dahmen et al. 2001, Wu et al. 2001, Taniguchi et al. 2002) was fairly well anticipated with the discoveries that APC and Axin proteins are necessary for effective β-catenin downregulation (Behrens et al. 1998, Hart et al. 1998). Except as just noted, an extensive body of knowledge has yet to surface regarding Axin mutation incidence in human cancer, including CaP. The importance of Axin in abrogating wnt/β-catenin pathway activity is seemingly reinforced by multiple independent investigations characterizing Axin-2 as a CRT target gene, which could therefore orchestrate some form of auto-feedback inhibition of β-catenin signaling (Yan et al. 2001, Jho et al. 2002, Leung et al. 2002, Lustig et al. 2002). Unlike certain other proposed CRT target genes, Axin-2 expression is unambiguously induced by mutant forms of β-catenin in CaP cells (authors’ unpublished data).

The F-box-containing protein β-TrCP1 targets phosphorylated β-catenin bound to the APC/Axin scaffold for ubiquitin modification, thereby initiating proteasomal degradation of the substrate (Hart et al. 1999, Kitagawa et al. 2002, Gerstein et al. 2002). These data culminate with
various modes of CaP (Gerstein et al. 1999). The interaction between certain WD40 domains of β-TrCP1 and β-catenin is dependent on the latter being phosphorylated by GSK-3β. Using its F-box, β-TrCP1 recruits to this complex an entourage of other factors such as Skp1 and Cul1 which mediate ubiquitin ligation of β-catenin. Gerstein and colleagues (2002) reported two β-TrCP1 alterations in CaP and propose that such changes could account for enhanced CRT in vivo. Their conclusion was based in part from certain of the afore-mentioned studies documenting that over-expression of a dominant-negative form of β-TrCP1, which lacks its F-box but retains β-catenin binding, effectively blocks β-catenin turnover. A more rigorous determination of β-TrCP1’s role in CaP is warranted for at least two reasons: (1) putative inactivating/dominant-negative mutations specifically affecting β-TrCP1 have not been reported elsewhere, perhaps implying an essential role for β-TrCP1 in other pathways that necessitate ubiquitination and (2) previous work has demonstrated that TSU cells – their non-CaP cell origin notwithstanding (see van Bokhoven et al. 2001) – do not exhibit a signaling profile consistent with endogenous β-catenin hyperactivity (Sasaki et al. 2000). The interstitial β-TrCP1 deletion in TSU cells (heterozygous Δ17–73), which should not impinge on the F-box (residues 148–192) (Margottin et al. 1998), might be predicted to lack dominant-negative effects on its wildtype counterpart. On the other hand, β-TrCP1 (Δ17–73) may be deficient in associating with β-catenin, as the former’s amino-terminal region may collaborate with its WD40 domains to bind β-catenin (Kitagawa et al. 1999). An incapacity to complex β-catenin most likely does characterize the other alteration (metastatic tumor) uncovered by Gerstein et al. (2002) which creates a nonsense mutation at position 212 of β-TrCP1, hence eliminating the WD40 domains. Definitively, an important inquiry will be to determine the extent to which β-TrCP1, which is incompetent in β-catenin binding, exhibits dominant-negative behavior or engenders haploinsufficiency with regard to β-catenin ubiquitination.

When is β-catenin alteration and elevated CRT selected in CaP – tumorigenesis, tumor progression or both? Taken further, could such molecular events serve as a predictor of tumor behavior (indolent vs aggressive)? Alterations in β-catenin and its regulators (e.g. APC and Axin-1 and -2) in human cancer generally occur in a mutually exclusive manner (Morin et al. 1997, Rubinfeld et al. 1997, Sparks et al. 1998, Miyaki et al. 1999, Wu et al. 2001), implying that β-catenin signaling can drive cell selection and consequent clonal expansion. This partitioning of pathway alteration may likewise apply to the etiology of β-catenin upregulation in CaP (Gerstein et al. 2002). These data would suggest that various modes of β-catenin upregulation contribute equally to oncogenesis; that said, we are intrigued by the possibility that the above-mentioned genetic abnormalities are not functionally equivalent, and can be uniquely associated with a certain subset or stage of tumor. For example, Samowitz et al. (1999) demonstrated that β-catenin missense mutations are more frequently detected in small colorectal adenomas (benign) than in large adenomas and adenocarcinoma – consider these data in light of evidence that functional APC loss is observed at roughly the same rate in both early and advanced forms of colorectal cancer (Powell et al. 1992). The potential absence of β-catenin regulatory domain mutations in AI CaP (generally metastatic lesions removed at autopsy), coupled with immunostaining analyses demonstrating nuclear β-catenin positivity in a moderate percentage of such lesions (~20%) (Chesire et al. 2002), implies that the above anomaly could also apply to CaP biology. The potential involvement of APC nullification and potentially other factors (e.g. Axin) in advanced, AI CaP could account for this interesting problem. This idea which we apply here to colon cancer and CaP should not, however, be confused with a similar but distinct phenomenon in which different mechanisms of β-catenin deregulation seem to be associated with one particular clinicopathological tumor phenotype (Nakatsuru et al. 1992, Wu et al. 2001, Ebert et al. 2002, Taniguchi et al. 2002). Importantly, although Gerstein et al. (2002) did partly address the concern that the APC alterations could have occurred in primary, AD disease, one cannot totally exclude this potential overlap. Results uncovered by de la Taille et al. (2003) speak to this issue: roughly 23% of primary, AD prostate tumors contain altered β-catenin localization by immunohistochemistry. Indeed, this frequency of putative β-catenin activation in AD disease is greater than the expected frequency (~5–10%) of direct β-catenin mutation in this same tumor class (AD), which likewise leads to altered localization in vivo.

The detection of direct β-catenin activating mutations predominantly in primary CaP may implicate this oncogene as a contributor to early tumor formation and/or less aggressive phenotype (i.e. lower chance of progression/mortality). In all three β-catenin mutation reports (Fig. 2a), only one alteration – interestingly, the only large interstitial deletion described – was found in advanced cases. The clonal nature of this deletion likely reveals its primary tumor origin; perhaps this result is of significance and demonstrates that removal of the entire GSK-3β phosphorylation domain represents a change functionally distinct from that imparted by missense changes. For instance, Samowitz et al. (1999) showed a preponderance of β-catenin missense changes in small colorectal adenomas compared with adenocarcinoma. In contrast, Iwao et al. (1998) likewise examined colorectal adenocarcinoma and found interstitial deletions exclusively, hinting that such changes are associated with more aggressive cancers. We should note that these two groups examined sporadic cases chosen at random, unlike similar studies which focused on certain genetic subsets of colorectal tumors (Sparks et al. 1998, Miyaki et al. 1999). Returning the focus to single amino acid changes, we hypothesize that such β-catenin alterations are selected in CaP tumorigenesis, but...
that a subset of primary tumors carrying these changes is less apt to progress. Our deduction takes into account multiple findings in other cancers which collectively give the impression that β-catenin involvement may be linked to less severe cancer phenotypes (Gamallo et al. 1999, Park et al. 1999, Saegusa et al. 2001, Taniguchi et al. 2002). The criteria from these reports that we used to formulate our opinion include disease stage, prognosis, and certain features of tumor differentiation – each of these parameters appropriately hinted at mitigated disease. Our general idea is schematically represented in Fig. 3. We stress here that this hypothesis currently suffers from an overall under-representation of advanced CaP cases (i.e. a higher number have to be examined) compared with the number of primary cases examined in β-catenin screening studies (see Fig. 2). This caution is compounded in light of the rather low mutation frequency observed in the latter. Although narrow in scope with regards to the above discussion, recent reports have shown that disruption of mutant β-catenin (A45S) expression in a colorectal cancer cell line does not substantially impinge on certain parameters of cell growth (Chan et al. 2002, Sekine et al. 2002). Therefore, such β-catenin changes may have diminishing oncogenic returns as selection of certain malignancies (or subtypes thereof) proceed.

An important inquiry will be to address the molecular basis underlying nuclear β-catenin localization in otherwise β-catenin mutation-free, AI CaP (~20%) (Chesire et al. 2002). The rate at which such altered localization occurs in advanced AI disease may be even greater: de la Taille and colleagues (2003) reported a rate of approximately 39%. As discussed beforehand, APC and Axin loss-of-function mutations may impart this manifestation of β-catenin hyperactivity; therefore, these genetic alterations may likewise contribute to nuclear β-catenin localization in AI prostate

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<th>Single cell</th>
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<td>level of differentiation:</td>
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**Genetic Alteration & Selection**

- β-catenin missense change
- β-catenin interstitial deletion
- APC loss or equivalent
- non β-catenin pathway mutations
- Prostatectomy
- Androgen Ablation
- Therapy

![Figure 3](image-url) **Figure 3** A very basic model for the contribution of β-catenin-associated genetic alterations on CaP progression. It draws from limited knowledge and certain clinicopathological data reported for other cancers bearing related alterations (see text). The top portion of the figure shows basic diagrams of prostate tumor cell morphology. Note that prostate tumor progression may tend toward anaplastic morphology. The cell shown within the vertical broken line represents a theoretical single cell which has survived androgen ablation therapy and will spawn an AI tumor. In the lower half, arrows simply denote a possible role for the listed mutation type in driving tumor progression. The relative time of prostatectomy in cases with AD, localized disease will vary.
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tumors. Gerstein et al. (2002) uncovered APC loss in 25% (3/12) of AI lesions, thus tentatively (i.e. more cases will need to be analyzed) implying that nullification of this tumor suppressor may be one causative factor responsible for nuclear β-catenin staining in AI disease. We have previously noted that, in certain AI cases, nuclear staining was correspondingly present in all lesions obtained at autopsy (i.e. clonal origin) – metastatic and localized (Chesire et al. 2002). Despite these data, the actual timing for selection of APC loss or other relevant genetic changes in CaP (AD vs AI disease) remains unanswered and thus merits further investigation (Fig. 3). As alluded to above, a hint to answering this question may be offered by the finding of abnormal β-catenin staining in ~23% of primary CaPs (de la Taille et al. 2003); here, the unknown lesions (not including presumptive β-catenin mutation) did occur in localized disease, but the extent to which these lesions would promote further tumor advancement is unknown. The findings of de la Taille and colleagues (2003) of putative β-catenin activation in ~23% and ~39% of primary and advanced tumors, respectively, could suggest that the unknown genetic changes do confer some sort of tumor growth advantage. In reference to APC, our discussion has been based on the assumption that nuclear β-catenin activity is the selected event triggered by APC loss-of-function mutation. Therefore, it is worth noting that APC exhibits various functions aside from that of down-regulating β-catenin, such as a positive role in chromosomal stability (Fodde et al. 2001, Kaplan et al. 2001); indeed, the selected phenotype for CaP progression that could result from APC loss may not be β-catenin activity but, rather, chromosomal instability.

More in vivo analysis is essential to clearly define the selected modes of β-catenin pathway activation during the natural history of CaP. Above, we drew attention to work by Gounari et al. (2002) characterizing transgenic mice which were engineered to express mutant β-catenin in the prostate. It is germane to mention here that, in light of the preceding discussion, the mutant species of β-catenin in the Catnb+/lox(ex3) mice lacked the entire GSK-3β phosphorylation domain. That these transgenic animals developed PIN-like lesions is significant: PIN is likely the precursor to CaP; therefore, aberrant β-catenin upregulation alone induces that new growth which is associated with early disease. Gounari and colleagues (2002) described areas of squamous metaplasia within the PIN-like lesions, although less so compared with that observed in other organs of the mice. This result concurs with other related transgenic reports, insofar that transdifferentiation and anachronous differentiation features often characterize those new growths induced by mutant β-catenin (Gat et al. 1998, Imbert et al. 2001, Miyoshi et al. 2002a,b). Further, that the PIN-like lesions were not reported to progress to full-blown carcinoma finds direct analogy (Cadoret et al. 2001, Harada et al. 2002, Miyoshi et al. 2002a,b). These data altogether suggest that β-catenin deregulation, in playing the lead role in a single hit model of carcinogenesis, is generally not sufficient to instigate malignancy. But these data are consistent with evidence that this pathway may be a major protagonist in early tumor formation (Kinzler & Vogelstein 1996, Samowitz et al. 1999). In conclusion, future experiments to introduce additional mutant alleles (‘genetic hits’) to the Catnb+/lox(ex3) mice should be insightful.

Potential β-catenin pathway cross-regulation in the prostate

Underlying CaP tumorigenesis and progression are a number of signaling and metabolic mechanisms, some of which may be unique to this tumor. Although far less is known on the subject, these same pathways are probably engaged in multiple facets of normal prostate physiology. Many of the ‘usual suspects’ (e.g. p53, pRB, c-myc, etc.) behind human cell transformation are similarly implicated in CaP pathogenesis (Isaacs et al. 2002). Among the long list of well-characterized oncogenes and tumor suppressors, those most commonly impacting CaP pathogenesis and that are of interest in this review are AR, PTEN, and E-cadherin. These molecules orchestrate pathways which may interact with – undoubtedly this is the case with E-cadherin – and therefore influence the effects of β-catenin activation in prostate cells. Here, we review relevant aspects of these prominent CaP pathways in order to provide a mechanistic backdrop for comprehending what might be prostate-specific β-catenin function (refer to Fig. 4 for overview).

Androgen receptor

Despite successes in curbing AD disease by hormone ablation therapy, effective treatment of AI tumors does not exist. That said, determining AR function is a central challenge in understanding CaP, as its expression is maintained almost without exception during tumor progression from androgen dependence to androgen independence (Hobisch et al. 1995, Balk 2002). Further, recent in vitro data (Zegarra-Moro et al. 2002) suggest that this transcription factor is critical for prostate cell survival in the androgen-scarce milieu generated by androgen ablation therapy. The AR, a member of the nuclear receptor family (Balk 2002), converts from a transcriptionally quiescent state to one that is active upon ligand binding (Masiello et al. 2002, Shang et al. 2002). In a homodimeric fashion, ligand-bound AR binds to androgen-response elements present in promoters of target genes, either upregulating or repressing gene expression. For the purposes of this review, we only point out that AR contains two general transactivation regions, the amino terminus and the ligand-binding domain (LBD) which both recruit transactivating factors (MacLean et al. 1997). Schemes for CaP cell acquisition of androgen independence invoke the
Figure 4 Pathways which may be linked to wnt/β-catenin/TCF signaling (detailed in Fig. 1). Each of the three pathways described in this figure is demarcated by a broken line and numbered. The free pool of β-catenin represents newly expressed molecules that can participate in different complexes (trace the heavier broken lines to different complexes as marked by arrows). The free pool also contains, hypothetically speaking, β-catenin derived from alternative sources as appropriately shown by arrows leading to the pool. (1) E-cadherin-mediated cell–cell adhesion. E-cadherin forms homotypic interactions with neighboring cells. To form a strong junction, auxiliary molecules α- and β-catenin must bridge the cytoplasmic domain of E-cadherin to actin microfilaments. β-Catenin is recruited to the junction, a process which may impact nuclear signaling by the former. Tyrosine phosphorylation of β-catenin in E-cadherin complexes weakens junction integrity and prompts disassembly. An important question is whether or not tyrosine-phosphorylated β-catenin contributes to the free pool and nuclear signaling. (2) Phosphoinositide-3 kinase (PI3-K)/Akt/PTEN pathway. Secreted protein growth factor (GF) ligands bind to receptor tyrosine kinases at the cell membrane, thereby inducing receptor dimerization and intrinsic tyrosine kinase activity (cytoplasmic domains). Receptor tyrosine kinases activate the PI3-K enzyme complex (p85 and p110), among many other enzymes. PI3-K phosphorylates phosphatidylinositol-4,5-diphosphate (PIP2) producing phosphatidylinositol-3,4,5-triphosphate (PIP3) which, in turn, is converted back to PIP2 via PTEN phosphatase activity. Akt kinase is activated by other kinases upon binding to PIP3, and then phosphorylates several different target proteins, including GSK-3β. Two eventualities (question marks) are shown in which Akt inhibition of GSK-3β may lead to suppression of the latter’s participation in β-catenin degradation. To elaborate, can Akt inhibit that GSK-3β species which is involved in the β-catenin destruction complex (?) or, after Akt downregulates free GSK-3β, can the latter participate in the β-catenin destruction complex (?)? Whether or not these mechanisms occur remains an enigmatic question. (3) AR/β-catenin pathway interactions. Free β-catenin may be able to interact with AR to augment ligand-dependent transcription. The source of β-catenin for this putative collaboration could be the cytoplasm and/or the nucleus. Unresolved issues related to the former and latter pool sources (question marks) are the possibility of AR-associated β-catenin nuclear import and AR-dependent repression of CRT, respectively. Note that the main ligand for AR is dihydrotestosterone (DHT).
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premise that AR regains transcriptional function despite low levels of circulating androgen. Putative mechanisms underlying this phenomenon have been elucidated (Culig et al. 1994, Wen et al. 2000, Gregory et al. 2001, Lin et al. 2001a, Linja et al. 2001): mutation of LBD, gene amplification, protein phosphorylation, and altered co-activator expression. The last of these mechanisms is of much interest for considering β-catenin involvement in normal and CaP physiology, both of which will be revisited in the next section.

Truica et al. (2000) were the first to demonstrate that β-catenin, especially that bearing activating mutations, is able to enhance AR transcriptional activity in a ligand-dependent fashion (Fig. 4, box 3). This observation came on the heels of the important discovery that β-catenin may similarly co-activate retinoic acid receptor (RAR) transcriptional function (Easwaran et al. 1999). More recently, Palmer et al. (2001) have demonstrated that this relationship also exists between β-catenin and vitamin D receptor (VDR). These ‘alternative’ modes of β-catenin nuclear function comprise a very small set of circumstances in which β-catenin is found to transactivate a DNA-binding transcription factor apart from TCF/LEF (Gallet et al. 1998, Zorn et al. 1999). Independent reports, largely in vitro, have since verified the original findings of β-catenin synergy towards AR activity (Chesire et al. 2002, Mulholland et al. 2002, Pawlowski et al. 2002, Yang et al. 2002, Song et al. 2003). Does this phenomenon encompass other nuclear hormone receptors besides AR, RAR, and VDR? In considering certain of the afore-mentioned studies, one may conclude that β-catenin most likely does not cross-regulate estrogen receptor, progesterone receptor (PR) or glucocorticoid receptor (GR).

Regarding the mechanism behind β-catenin-mediated stimulation of AR, multiple lines of evidence converge on the same conclusion that both molecules may interact and, in order to do so, require ligand. The results of several techniques provide this general assessment: co-immunoprecipitation, GST–fusion protein interaction, yeast and mammalian two-hybrid analyses, nuclear colocalization assays, and other procedures (Truica et al. 2000, Mulholland et al. 2002, Pawlowski et al. 2002, Yang et al. 2002, Song et al. 2003). Certain experiments demonstrating β-catenin/nuclear hormone receptor (RAR and VDR) interaction may lend support to this conclusion (Easwaran et al. 1999, Palmer et al. 2001). Immuno-colocalization work suggests that, regardless of the nature of putative AR/β-catenin complex formation, β-catenin recruitment to AR entails its own nuclear import, part of which may be facilitated by AR. The afore-mentioned studies also provide insight into the domains of AR and β-catenin that are required for mutual complex formation (AR domains are reviewed in Fig. 5); since a diverse array of experimental readouts were employed amongst these investigations, we offer only limited speculation on this matter. The data culminate with the idea that the AR LBD plays a central role in recruiting β-catenin to the proposed complex, although discrepancies do exist within the data. The ligand-dependent nature of β-catenin transactivation of AR-mediated gene transcription may corroborate this conclusion. This information is very interesting: despite a relatively high degree of conservation among the LBDs of AR, GR and PR, only AR LBD is functionally linked to β-catenin function. Further, compared with its homology to GR and PR, AR shares far less homology with RAR and VDR, both of which can interact with β-catenin. Perhaps reflecting these seeming ambiguities are some of the data, but not all, provided by Song et al. (2003) that would functionally separate the AR-enhancing activity of β-catenin alone from that potentiated by p160 nuclear receptor co-activators. Taking all this information into account, additional studies similar to those listed in Fig. 5 are called for in order to confirm and improve upon these interaction data (e.g. see fine interaction mapping reported by Song and colleagues (2003)).

Work by the same laboratories (Mulholland et al. 2002, Pawlowski et al. 2002, Yang et al. 2002) has identified the region of β-catenin involved in AR complex formation. While all these groups did identify the general area necessary for AR association – the armadillo repeats – only Yang et al. (2002) performed deletion analyses to hone in on the particular repeat region. Using a combination of yeast two-hybrid (certain β-catenin constructs were captured with AR LBD as bait), GST-pulldown, immuno-colocalization, and AR co-activation analyses, they mapped the interaction interface to armadillo repeats one through six – there are 12 in total. As we judge from reading their report, less time was required by Pawlowski et al. (2002) to implicate essentially the same region, as it was encoded by a cDNA clone acquired from their initial yeast two-hybrid experiments. These data too, just as those pertaining to the relevant AR region, would likely benefit from more evaluation, especially since the armadillo repeats are essential for mediating β-catenin engagement with several other regulatory and structural factors (Miller et al. 1999). Three major components of which – E-cadherin, APC and TCF/LEF – all impact on β-catenin function and metabolism. LxxLL protein motifs are present in most co-activators of nuclear steroid receptors – β-catenin contains five such motifs – and mediate interactions between these two classes of molecules. Intriguingly, certain results reported by Yang et al. (2002) and Song et al. (2003) suggest that these motifs are not obligatory for β-catenin/AR interaction. Deficits in knowledge as exemplified here are probably greatly under-appreciated, thus our current working hypotheses regarding β-catenin/AR interaction may underestimate its actual level of complexity.

Given the likelihood of β-catenin/AR pathway interaction, an obvious question arises: is there any form of reciprocity with respect to the effects of AR signaling on β-catenin input to other pathways, the most ostensibly of which being TCF target gene transcription (CRT)? There are
Figure 5 Multiple AR fragments tested by three individual groups which are instructive for defining a putative β-catenin-binding region in AR. This figure is purposely incomplete with regards to all those regions assayed by the studies. Listed with each bracket is reference to the portion of AR found to interact with β-catenin, followed by the experimental technique utilized by the authors and a plus or minus sign denoting putative β-catenin-binding status (+, evidence for interaction; −, no evidence for interaction). Note that apparent inconsistencies do exist and probably are related to the use of different techniques by independent laboratories. Numbers indicate amino acid residue position. The AR is a polymorphic protein, therefore its actual length varies. In reference to data reported by Mulholland et al. (2002), residue number 548 is treated as residue 558 for ease of comparison with the other studies. The darker region between residues 624 and 676 represents the hinge domain which contains nuclear localization signals. NtD, amino-terminal domain; Ct, carboxy terminus; Conf. yeast 2-hyb, confirmatory yeast two-hybrid analysis using the listed fragment as bait; Imm. coloc., immuno-colocalization of fragment with β-catenin in the nucleus; GST-fus. prot. int., GST–AR fusion protein interaction with β-catenin armadillo repeats; Co-fract. nuc., nuclear co-fractionation of the AR fragment and over-expressed β-catenin; AR co-act. assay, AR fragment transcriptional co-activation upon β-catenin over-expression. Mamm. 2-hyb, mammalian two-hybrid analysis using listed fragment as bait and VP16-β-catenin fusion protein. Note that in the report by Song et al. (2003), it is stated in the text that mammalian two-hybrid analysis showed no interaction between β-catenin and AR fragment 1–503.

Of interest would be to determine if other nuclear receptors such as PR or GR similarly repress CRT. If the above working hypothesis holds true, we conjecture that CRT repression would not occur due to the lack of evidence linking PR/GR to β-catenin; indeed, results backing this notion demonstrate that GR is not capable of mediating ligand-dependent repression of CRT (Song et al. 2003). The exact mechanism for this phenomenon of CRT repression may quite possibly be unrelated to the above-stated β-catenin availability hypothesis, but rather may involve some other factor or process. Although it may go without saying, the experiments used to dissect the heretofore unrecognized AR/β-catenin interaction and reciprocal cross-regulation were necessarily reductive, thereby probably eliminating several variables of relevance in vivo.

PI3-K/Akt/PTEN signaling pathway

The PTEN phosphatase is a tumor suppressor involved in a wide array of human malignancies, including CaP (Ali et al. 1999, Simpson & Parsons 2001). Briefly, PTEN downregul-
ates PI3-K intracellular stimulation by hydrolyzing the 5-phosphate group of PIP_2, generating PIP_3 (Fig. 4, box 2). Inhibitors of PI3-K elicit the same biochemical response, albeit through indirect means. With loss of PTEN function, PIP_3, accrues and prompts elevated Akt kinase activity. There are certain oncogenic events in CaP that may act to override wildtype PTEN inhibition and impel aberrant Akt kinase activity (e.g. receptor tyrosine kinase upregulation). Numerous different outcomes of Akt activity are probably associated with the molecular etiology of CaP, but perhaps the most tissue-specific mark of its activity in prostate cells is its putative ability to directly phosphorylate AR (Wen et al. 2000, Lin et al. 2001b).

Here, we approach the intriguing idea that the Akt oncprotein positively modulates β-catenin signaling function during prostate tumor progression, through both AR-dependent and -independent means. The former mode invokes the potential co-activating function of β-catenin towards AR (above), whereas the latter simply refers to those transcriptional processes effected by β-catenin through other transcription factors (e.g. TCF/LEF); indeed, both processes would likely be interrelated. The mechanism proposed to underlie both these phenomena is founded on the multifaceted nature of GSK-3β activity – it is a key regulator in multiple pathways, namely those of glycogen metabolism and wnt signaling (Cohen & Frame 2001). GSK-3β, aside from its role in downregulating β-catenin (see Figs 1 and 4), is targeted for inhibition by Akt-mediated phosphorylation. Thus an enticing question is broached: can nuclear β-catenin signaling be upregulated by Akt stimulation in a manner independent of wnt stimulation? This scenario could entail processes previously implicated in CaP pathogenesis such as receptor tyrosine kinase pathway activation (Culig et al. 1994, Wen et al. 2000) or, perhaps more commonly, abrogated PTEN function. We have been unable to demonstrate any functional link between PI3-K/Akt/PTEN pathway activity and β-catenin nuclear signaling (Chesire et al. 2002, authors’ unpublished data). In contrast, work in other laboratories has shown a positive link between these two events (Persad et al. 2001, Sharma et al. 2002). Sharma et al. (2002) posit that augmented AR transcriptional activity that occurs concomitantly with elevated Akt activity basically represents a co-activation event: Akt checks GSK-3β function, thus prompting β-catenin stabilization and nuclear collaboration with AR. Consistent with our findings, they mention failure to show coincident enhancement of β-catenin/TCF transcriptional activity (CRT) in their model system. However, we remain intrigued by this lack of change in CRT, given the presumptive increase in nuclear β-catenin invoked by their experiments. Although this negative outcome may be related to their use of LNCaP cells, which are recalcitrant to CRT induction as measured by reporter analyses, it is difficult to reconcile these reports of unaltered CRT with those of Persad et al. (2001), who did positively correlate Akt-mediated GSK-3β suppression with upregulated CRT.

The wide variety of results discussed here and elsewhere (Yuan et al. 1999, Ding et al. 2000) seem to obscure any comprehension of the degree to which PI3-K/Akt/PTEN and β-catenin pathways interact. That said, since we were unable to prove in our own hands that Akt activity inputs to CRT, attention is purposely drawn to data that favors the cross-regulation hypothesis (Ding et al. 2000, Cohen & Frame 2001, Weston & Davis 2001). These papers (and references therein) propose that both the wnt and PI3-K pathways can inhibit GSK-3β enzymatic activity in vitro, but only the former is similarly able to do so under in vivo conditions as it relates to β-catenin regulation. This discrepancy may be due to certain conformational parameters that limit Akt activity to regulation of ‘free’ GSK-3β, but not that which is participating in β-catenin regulatory complexes (Fig. 4, box 2). The necessity for further inquiry into whether or not these two pathways intersect is obvious, especially in considering matters of cell- and tissue-type specificity; that said, we relate here a totally different vantage point from which to evaluate the action of this putative phenomenon in CaP. PTEN loss-of-function is predicted to occur in a substantial fraction of advanced tumors (Simpson & Parsons 2001) – such tumors would be expected to exhibit elevated Akt kinase activity and, therefore, might be expected to contain activated β-catenin. If the detection of nuclear β-catenin is a reliable measure of its ‘oncogenically relevant’ activity (i.e. of consequence to tumor selection) in advanced lesions (~20%) (Chesire et al. 2002), then there does not seem to be any noticeable correlation between β-catenin activation and PTEN downregulation in CaP.

E-cadherin

Adherens junctions play an important role in epithelial cell–cell adhesion, and their breakdown plays a critical role in cancer cell invasion and metastasis. E-cadherin is the central structural component of these junctional complexes, consisting of an extracellular domain responsible for homotypic interactions with neighboring cells, a transmembrane domain, and a cytoplasmic domain (Takeichi 1991). The last of these is anchored to the actin cytoskeleton via bridging molecules – β- and α-catenin (Fig. 4, box 1). Aside from its structural functions, the β-catenin homologue plakoglobin (γ-catenin) is capable of nuclear signaling (Simcha et al. 1998). A decisive mark during epithelial-to-mesenchymal transition, which is heavily involved in embryonic development and can contribute to certain aspects of oncogenesis, is downregulation of E-cadherin expression (Battle et al. 2000, Cano et al. 2000). Altered E-cadherin expression occurs frequently in CaP (50%) and correlates with certain clinicopathological parameters (Umbas et al. 1992). Although complete loss of E-cadherin expression is linked to the natural

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history of certain cancers (e.g. gastric), it is rarely permanently lost in prostate tumors (De Marzo et al. 1999b). Adherens junction formation affects the intracellular balance of β-catenin (soluble vs insoluble), thus invoking a tantalizing question: could greater comprehension of E-cadherin expression be of any value in estimating β-catenin signaling during tumor progression?

The interplay between E-cadherin and β-catenin and its effects may conform well within a popular hypothesis for the basis of cell–cell contact-mediated growth suppression. This theory implicates regulatory factors and their fluctuation between different subcellular compartments – here, the cytoskeleton and nucleus are probably key. Perhaps altered E-cadherin expression promotes cell selection by permitting not only loss of adhesion, but also aberrantly high β-catenin signaling (Fig. 4, box 1). Note that this proposal (i.e. β-catenin instigating tumor progression) may seem to contradict our previous conjecture on the effects of nuclear β-catenin in CaP (refer to Fig. 3) but, again, the cellular context in which these different phenomena occur remains a very important consideration. Earlier experiments demonstrated a negative effect for cadherin molecule expression on β-catenin specification activities in Xenopus embryos (Heasman et al. 1994, Fagotto et al. 1996). These insights, together with a heightened appreciation of E-cadherin involvement in neoplasia, may have further drawn attention toward understanding the effects of cadherin expression on β-catenin’s nuclear signaling capacity (Sadot et al. 1998, Caca et al. 1999, Sasaki et al. 2000, Gottardi et al. 2001, Yang et al. 2002). Some of these reports suggest that β-catenin nuclear activity is negatively regulated by cadherin expression; in general, however, this downregulation was only evident in those model systems employing aberrant β-catenin metabolism.

In addition to speculating on the effects of cadherin expression alone, levels of free β-catenin available for nuclear signaling may also be a function of junctional complex stability. Junction disassembly can occur upon tyrosine phosphorylation of constituent β-catenin, the latter process being induced upon cell stimulation by secreted growth factors (e.g. epidermal growth factor) which prompt receptor tyrosine kinase ligation and activation of intracellular tyrosine kinases (e.g. src) (Daniel & Reynolds 1997; Fig. 4, box 1). Although soluble β-catenin does increase after tyrosine phosphorylation, there is no clear evidence that this result alone incurs elevated β-catenin/TCF transcriptional activity (CRT) (Playford et al. 2000, Kim & Lee 2001). However, such an increase of soluble β-catenin coupled with GSK-3β inhibition can stimulate CRT upregulation (Playford et al. 2000). Here, the extent to which this observation would implicate PI3-K signaling in this general scenario should also be considered.

In consideration of our unpublished data and certain afore-mentioned studies, we are led to propose that cadherin expression modulates induced, but not basal, CRT and other β-catenin nuclear activities. This argument supposes that, even in the absence of adherens junctions, β-catenin which has not been stabilized – for example, by wnt signaling – is constitutively ubiquitinated and degraded. However difficult to devise or interpret, more in vivo-related studies are paramount to test this hypothesis. Cadherin-related events may certainly impact on β-catenin nuclear function, but such effects may be only transient in nature, never yielding a tumor with constitutively high nuclear β-catenin activity. Perhaps such a short-lived phenomenon accounts for the in vitro findings which inversely correlate cadherin expression with β-catenin signaling. Despite these and other caveats, an intriguing idea is evoked from our proposal: if wnt signaling is involved in prostate physiology (e.g. morphogenesis), could it be augmented with concurrent stimulation by growth factors that promote adherens junction disassembly? For example, tyrosine kinase activity is partly responsible for initiating those morphological changes preceding mitosis (e.g. cell rounding); therefore, wnt/β-catenin signaling under these circumstances may be substantially augmented and so able to affect mitosis (see cell cycle analyses in Orford et al. 1999). In a final digression, worth mentioning here is work showing that caveolae and a major constituent protein thereof, caveolin-1, may sequester β-catenin complex and consequently downregulate CRT (Galbiati et al. 2000). Interestingly, caveolin-1 has been implicated in other signaling pathways which are of direct relevance to CaP (Tahir et al. 2001).

Physiological aspects of β-catenin/AR pathway interaction
AR continues to be a major focus for probing aspects of prostate biology. Therefore, in this final section, we will speculate on the potential nature of AR and β-catenin pathway cross-talk in the prostate. Our objective is to present certain properties of AR and β-catenin physiological function that may be inter-dependently linked insofar as their ultimate effect on prostate biology is concerned. Therefore, attention will not be drawn towards specific AR and β-catenin/TCF target genes that may be of any consequence to the physiological processes discussed below.

Prostate formation
The extent to which restoring testosterone to castrated animals, which induces regeneration of prostate glands from an involuted state (see Fig. 6a), represents an adequate surrogate model for studying prostate morphogenesis is debatable. Regardless, both these growth-related processes (experimental prostate regeneration, prostate development) share common features through their mutual dependence on a presumptive prostate stem cell compartment and their ability to create a mature,
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**Figure 6** (a) Schematic depicting a cross-sectional view of a prostatic acinus and its general morphology throughout a testosterone (T) cycling experiment. Three cell compartments are listed: mesenchymal, basal epithelial, and luminal epithelial (secretory). Note that luminal (AR+) cells of the mature gland generally bear tall, columnar morphology, but adopt a flattened, non-secretory phenotype under androgen withdrawal (atrophic gland). Basal cells (AR +/-) maintain a flattened, non-secretory phenotype and are generally recalcitrant to androgen fluctuation. The mesenchymal cell compartment consists of fibroblast and myoepithelial cells. The bottom/middle schematic represents a still-frame of cellular activity during testosterone-induced prostate re-growth, which is accompanied by cell division to replenish luminal epithelia. After 24 h re-administration of testosterone to castrated animals, β-catenin displays a heterogeneous nuclear localization profile which persists through to at least 72 h post-treatment. Ki67 is a proliferation marker. Nuc. β-cat, nuclear β-catenin. (b) Mesenchymal-to-epithelial and epithelial-to-epithelial signaling which could play a role in β-catenin nuclear localization in luminal cells during androgen-induced prostate regeneration. Arrows leading from certain compartments (mesenchymal or luminal) denote androgen-induced secretions therefrom. The left-hand cell is shown to receive more stimulation (e.g. wnt factors potentially), thereby augmenting its level of β-catenin stabilization and nuclear translocation (compared with the right-hand cell). This differential in β-catenin stabilization and locale is represented by various shades of gray (see inset triangular scale). The amount, targeting, source, and relative effect on β-catenin metabolism by the depicted secretions are currently speculative. Strong β-catenin immunostaining is observed at adherens-mediated cell–cell junctions (dark rectangle).
functioning gland. Androgen cycling experiments could, therefore, prove useful for determining a role for β-catenin signaling in processes underlying prostate formation. Similar androgen withdrawal/replacement studies using the rat ventral prostate model were undertaken in our laboratory and demonstrated β-catenin nuclear localization during testosteron-induced gland re-growth (Chesire et al. 2002; see Fig. 6a for composite result). That this staining was observed in proliferating luminal epithila invokes an interesting question: could these particular cells represent a transient amplifying population? Staining these specimens for keratins and/or cell cycle-dependent kinase inhibitor p27 could furnish an answer (De Marzo et al. 1998a). If this idea is confirmed, one could possibly hypothesize that β-catenin signaling is in some way linked to that cell compartment from which CaP precursor lesions are believed to arise (De Marzo et al. 1999a), a notion already conveyed by a transgenic study (Gounari et al. 2002, see above).

An obvious and interesting question is raised after mulling over the evidence for β-catenin activation in the above-mentioned experiment: during androgen-induced prostate regeneration, what is the causal relationship between nuclear β-catenin and growth effects (i.e. cause or effect)? Assuming β-catenin signaling plays an active role in this process, culling out the mechanism(s) responsible for its nuclear localization in this model could be insightful for evaluating those analogous situations which may occur during actual prostate development. Those data (Gavin & McMahon 1992, Bradbury et al. 1995, Brisken et al. 2000) implicating progesterone-induced wnt factor expression during particular morphogenic processes of the mammary gland might imply that comparable wnt/β-catenin-associated signaling schemes may similarly be employed in prostate. Both paracrine and autocrine cell signaling processes are invoked by this sort of mechanism; for example, downstream an androgen stimulus in prostate, wnt secretion by epithelial and mesenchymal cells could be involved in targeting β-catenin upregulation in the epithelium (Fig. 6b). Supporting this mindset are precedents for both mesenchymal-to-epithelial (paracrine) and epithelial-to-epithelial (paracrine/autocrine) cell signaling events that are induced by prostate androgen exposure (Hayward et al. 1997). Taken from another angle, nuclear β-catenin localization upon testosterone re-administration to castrated animals may be a direct consequence of its putative nuclear import by AR (Mulholland et al. 2002, Pawlowski et al. 2002, Yang et al. 2002) (Fig. 4, refer to box 3). Although these groups documented immunocytochemical evidence for this ligand-dependent event, we have been unable to detect this form of β-catenin nuclear translocation insofar as our chosen androgen treatment time-course is concerned (20–24 h) (Chesire et al. 2002, authors’ unpublished data). Irrespective of this discrepancy, as β-catenin nuclear staining in the rat model was heterogeneous and anticipated to be downregulated upon duration of organ regeneration despite the continued presence of AR and ligand (Fig. 6a, refer to ‘Mature Gland’), we suggest that putative AR-dependent nuclear localization of β-catenin cannot by itself account for this in vivo finding.

Evaluating the phenotypic consequences of presumptive β-catenin transcriptional activity in studies of prostate regeneration could elucidate its signaling role in natural prostate growth and homeostasis. At present, little is known about the nature of β-catenin signaling in the normal prostate; indeed, β-catenin nuclear involvement has only been implicated in two transcriptional processes: TCF and AR target gene expression. As already discussed, β-catenin/TCF activity (CRT) during tissue development could potentially facilitate growth and differentiation – in the prostate, these physiological properties are similarly associated with ligand-dependent AR function. Hence, it may be judicious to consider CRT and AR target gene expression together in surmising nuclear β-catenin’s input to prostate development, especially since the former two transcriptional processes are likely interdependent. To this end, we briefly highlight here three phenomena which have been covered in this review: (1) β-catenin enhancement of AR activity, (2) CRT, and (3) AR pathway repression of CRT. Since the true nature of each of these phenomena in vivo must still be tested, we only present a limited perspective on how they might operate in prostate tissue, given realistic circumstances of androgen presence or absence (see Fig. 7, ‘Normal Prostate’).

Prostate cancer

Aside from a putative role in normal prostate tissue, androgen-related modes of β-catenin/AR pathway cross-talk may play an active role in CaP progression. Because a major turning point in CaP progression is the acquisition of an AI phenotype born from the selective pressure of androgen ablation therapy, it is reasonable to speculate on the nature of these pathway interactions from the perspective of their putative involvement in AD and AI disease (see Fig. 7, ‘Prostate Cancer’). One may question if β-catenin-mediated enhancement of AR activity contributes to this transition (AD-to-AI disease) which, for the most part, probably relies upon AR regaining transcriptional function. We pose this uncertainty in light of the ligand-dependent manner in which β-catenin is known to stimulate AR. It will be of interest therefore to determine under which oncogenic settings of androgen-independent AR upregulation β-catenin may stimulate AR signaling. As one possible example, AR altered in ligand binding specificity may have the capacity to draw on β-catenin-mediated enhancement. A related question will be to determine if any forms of androgen-independent AR activity are capable of repressing CRT, apart from that of AR activity associated with promiscuous ligand binding.

A subset of AI lesions may contain AR activity that does not recruit β-catenin-mediated stimulation. We conjecture that under such circumstances, CRT and its effects may be more potent due to relief of AR-mediated CRT repression.

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Figure 7  All other variables being the same, this chart attempts to summarize putative androgen-related aspects of \( \beta \)-catenin signaling through either TCF (for CRT) or AR. For the purposes of presentation, we are only considering those circumstances in which \( \beta \)-catenin may be stabilized and localized to the nucleus (certain of these scenarios may never occur). Arrows (see key, bottom left) denote the relative effect of \( \beta \)-catenin activity on TCF or AR function. Comments listed below the arrows for each scenario refer to the putative effects of \( \beta \)-catenin on the appropriate activity. Cellular context and the degree to which transcription factors (TCF or AR) recruit \( \beta \)-catenin activity in vivo are two important variables which are difficult to adequately present here.

This idea suggests that a portion of hormone-refractory tumors containing nuclear \( \beta \)-catenin staining (Chesire et al. 2002) bears elevated CRT – the effects of CRT therein are unknown. Early evidence in vitro implies that one effect of CRT in advanced CaP could impart increased resistance to apoptosis (de la Taille et al. 2003). On the other hand, one might hypothesize that activated \( \beta \)-catenin was selected in these hormone-refractory tumors by virtue of its co-stimulatory activity towards promiscuously liganded AR (mutant LBD); interestingly, the frequency of such LBD alterations in advanced CaP is roughly comparable to that of \( \beta \)-catenin nuclear positivity.

Concluding remarks

The role that \( \beta \)-catenin signaling plays in human CaP remains far from completely understood. We have approached this problem mainly from the perspective of understanding \( \beta \)-catenin nuclear involvement in CaP, paying close attention to its regulation of TCF- or AR-mediated transcriptional pathways. Given the current understanding of molecular CaP etiology, we suggest that androgen signaling is one pathway in particular that may help generate the complexity likely characterizing \( \beta \)-catenin signaling in CaP. Further comprehension of these and other forms of \( \beta \)-catenin signaling in CaP pathogenesis, as well as their phenotypic effects, will require much investigation and could provide the rationale for future modes of prostate tumor therapy. Advances in molecular tumor biology should continue to reveal the tissue-specific nature underlying certain signaling mechanisms at work in malignancy. Given this assessment, obtaining a useful understanding of \( \beta \)-catenin involvement in CaP may necessitate greater appreciation of \( \beta \)-catenin signaling function in normal prostate tissue.
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