Wnt and SHH in prostate cancer: trouble mongers occupy the TRAIL towards apoptosis

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Abstract

Prostate cancer is a serious molecular disorder that arises because of reduction in tumour suppressors and overexpression of oncogenes. The malignant cells survive within the context of a three-dimensional microenvironment in which they are exposed to mechanical and physical cues. These signals are, nonetheless, deregulated through perturbations to mechanotransduction, from the nanoscale level to the tissue level. Increasingly sophisticated interpretations have uncovered significant contributions of signal transduction cascades in governing prostate cancer progression. To dismantle the major determinants that lie beneath disruption of spatiotemporal patterns of activity, crosstalk between various signalling cascades and their opposing and promoting effects on TRAIL-mediated activities cannot be ruled out. It is important to focus on that molecular multiplicity of cancer cells, various phenotypes reflecting expression of a variety of target oncogenes, reversible to irreversible, exclusive, overlapping or linked, coexist and compete with each other. Comprehensive investigations into TRAIL-mediated mitochondrial dynamics will remain a worthwhile area for underlining causes of tumorigenesis and for unravelling interference options.

Introduction

It is well known that local therapy to a primary tumour is unsuccessful when it has metastasized. This resistance

against broad range of therapeutic interventions is because of 'recalcitrant microenvironment' that drives carcinogenesis. Nevertheless, this view is now being challenged by new laboratory and clinical data. It is attractive to speculate that some primary tumours release factors that enter the circulation which, by mobilizing cells from the bone marrow, enhance responsiveness of distant organs to metastasis. Clinical observations on prostate cancer are all unswerving in the concept that rational drug design directed against the primary tumour might also suppress progression of existing metastases.

Signal transduction is transmission of information concerning from compositional state of the extracellular environment to intracellular cytoplasm, that generates morphological or genetic responses. Moreover, this can also be a communication of the state of supramolecular structures, such as plasma membrane or chromatin, in the cell. This information is relayed through and by the cytoplasm is modulated by transitions involving steady states of cytoplasm's intricate reaction network.

Innovative approaches in mammalian functional genomics are emerging through harmonization of high throughput technology and methods that allow manipulation of gene expression in living cells. Here, we elucidate application of multipronged approaches towards understanding biology, and provide an account of negative regulators offering opposition against mechanisms of TRAIL-induced apoptosis.

Prostate cancer proteome: many TRAILs lead to Rome

Tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) is a well-acclaimed death receptor ligand that has the potential to preferentially trigger apoptosis in malignant cells, sparing normal cells. Increasing evidence suggests that TRAIL-based therapeutics, together with recombinant TRAIL, TRAIL-receptor agonistic

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antibodies and TRAIL gene therapy, have impressively made their entry into clinical trials. However, TRAIL therapy is relentlessly challenged by cytoplasmic proteins, which either suppress apoptotic proteins or suppress expression of respective receptors of TRAIL, on the cell surface. Taking into consideration suppression or non-availability of DR4/5 on cell surfaces, recently, cisplatin and a potent platinum (IV) complex plus cationic amphipathic lytic peptides have been evaluated for enhancing availability of DR4/5 (1,2). Similarly, cisplatin has been used in combination with lexatumumab and resulted in upregulation of death receptors and suppression of anti-apoptotic proteins (3). Likewise, HGS-ETR2, an agonistic human monoclonal antibody, specific to TRAIL-R2, provides a potential therapeutic intervention against prostate cancer (4).

Shh signalling in prostate cancer

Sonic hedgehog (Shh) is the most extensively categorized of the three vertebrate Hedgehog homologues, and is necessary for appropriate embryonic development. Shh binds to its receptor, Patched (Ptch1), following de-repression of Smoothened (Smo). This drives activation of Gli2, which triggers transcription of target genes, primarily *Gli1* and *Ptch1*. It is significant to mention aberrant activation of components of Shh by stimulating or suppressing a broad range of target genes (5), and other transduction cascades may participate in development of prostate tumours (6). In accordance with the same approach, epidermal growth factor receptor (EGFR) and hedgehog cascades present a decisive role in prostate cancer progression and are refractory to clinical therapies with disease relapse (7).

Recent data suggest that autocrine Shh signalling is instrumental in driving prostate carcinogenesis and androgen-independent cancer progression (8,9). It is documented to be involved in transformation of prostate basal/stem cells into prostate cancer stem cells (PCSCs) (10).

The well-acclaimed effector of Shh signalling, GLI1, suppresses prostate cancer progression by acting as a co-repressor, to substantially block androgen receptor (AR)-mediated transactivation, (11). Hh signalling pathway is upregulated in epithelial cells of hormone-treated prostate cancer (HTPC) and hormone-refractory prostate cancer (HRPC) (12).

It is intriguing to note that Hh pathway activation in myofibroblasts alone is adequate to drive tumourigenesis. Keeping in view patients with an abundance of myofibroblasts in their biopsy tissues, anti-Hh therapy might prove to be an effective therapeutic intervention (13).

It has lately been found that Hh signalling mediates proliferation of prostate cancer cells by controlling stathmin1 expression. Stathmin1 is a microtubule-regulating

protein that has an imperative task in assembly and disassembly of the mitotic spindle. Stathmin1 is expressed in normal developing mouse prostate and in prostate carcinogenesis. Interaction between Hh and stathmin has been unravelled in treatment of cells with cyclopamine, which suppressed expression of stathmin. On a similar note, overexpression of Gli further confirmed escalation in expression of the stathmin (14).

It is well documented that Sonic hedgehog (Shh) and bone morphogenetic proteins (BMP-4, BMP-7) are expressed by urogenital sinus epithelium and mesenchyme and interestingly both signalling cascades exert reciprocal and coordinated effects on outgrowth of nascent prostate ducts. Robust expression of Shh in LNCaP xenografts enhances tumour growth. There is convincing evidence for enhanced expression of Noggin and BMP-7 mRNA in the stromal component of Shh overexpressing xenografts. BMP4/BMP7 counteracts uncontrolled cell proliferation; however, the effects are impaired in presence of Noggin (15). This crosstalk between two linear pathways needs to be explored to dismantle the complex web of regulators of prostate cancer.

Krüppel-like factor 6 (Klf6) is a member of a zinc finger transcription factor family acknowledged to participate in development and tumour suppression. It has recently been documented that Klf6-deficiency leads to escalated levels of hedgehog pathway components (Shh, Ptc, and Gli). This overexpression dampens their localized expression and enhances impaired lateral branching (Fig. 1) (16).

A further important interpretation is implication of Shh signalling in prostate cancer cells and pre-osteoblasts, on osteoblast differentiation, an essential process that governs new bone formation that also characterizes prostate carcinoma metastasis. It is appealing to note that SHH suppresses expression of osteoblast differentiation transcription factor Runx2 and its target genes, *osteocalcin* and *osteopontin*. This mechanism of suppression of Runx2 needs to be investigated to explore effects of this protein on cell type plasticity (17).

It is imperative to note that NF-kappaB binding sites in the human harbour the Shh promoter region that binds exclusively to NF-kappaB complexes. Moreover, NF-kappaB-modulated transcriptional activation of Shh is mapped to a minimal NF-kappaB consensus site at position +139 of the Shh promoter. The consequence of NF-kappaB activation is elevated Shh mRNA and protein expression *in vitro* and *in vivo* in a genetic mouse model of inducible NF-kappaB activity. However, an important piece of evidence is that NF-kappaB-mediated Shh expression promotes proliferation and confers resistance to TRAIL-induced apoptosis (18). Shh desensitizes cells to TRAIL-mediated apoptosis; however, underlying

Figure 1. Various Shh-mediated suppression and stimulation of target genes.

mechanisms also have recently been explored. Emerging data suggest that GLI proteins act as repressors and activators for death receptors and cFlip, respectively.

There is overexpression of GLI2 and concomitant high expression of caspase-8 inhibitor, cFlip as cFlip promoter harbours GLI2 binding sites. Therefore, suppression of GLI2 results in suppression of cFlip and subsequent sensitization of cells to TRAIL (19). Contrary to this, GLI3 acts as a transcriptional repressor of Death receptor 4 (DR4) as abrogation of GLI3 results in recapitulation of expression of DR4; however, cells reconstituted for GLI3 suppressed DR4 expression (Fig. 1) (20).

Small molecule inhibitors have gained attention of researchers. In support of this notion, SANT-2 was identified as a potent antagonist of Hh-signalling pathway. However, while comparing various derivatives of SANT-2, it was observed that TC-132 was predominant in efficacy and displayed a 16-fold higher Hh-inhibiting activity than observed for the plant alkaloid cyclopamine (21).

Crosstalk between Wnt and TRAIL

The Wnt family of secreted ligands operates through multiple receptors, to modulate discrete intracellular linear and integrated signalling pathways in embryonic development, in adults and in prostate cancer progression. Attachment of Wnt to Frizzled family receptors and to low density lipoprotein receptor-related protein 5 (LRP5) or LRP6 co-receptors, triggers cytoplasmic Wnt- β catenin transduction cascade, which mediates β catenin stability and context-dependent transcriptional responses. An interesting feature is that stabilized -catenin forms multicomponent transcriptional complex with LEF/Tcf and activates downstream targets such as c-Myc (Angers and Moon, 2008). In the following section, we will discuss some recent findings with reference to Wnt in prostate cancer. We will also discuss models for Wnt signalling and probable interaction between Wnt and TRAIL, as unravelled by worldwide studies. We will outline some therapeutic interventions for targeted inhibition of Wnt signalling.

Wnt signalling is negatively regulated by multiple proteins (Axin, APC, GSK, CK, β TRCP), which coordinate and orchestrate degradation of β -catenin. Recent data suggest a potential prognostic role of APC rs3846716 GA/AA genotype on PSA recurrence, after radical prostatectomy (RP) (22). Protein tyrosine kinase 6 (PTK6) negatively regulates β -catenin/TCF-mediated transactivation in prostate cancer cells (2). The upcoming section highlights that Wnt signalling activates/stimulates proteins and respective target genes, which drive prostate cancer progression. We will outline various Wnt isoforms (Wnt5a and Wnt 11 positively regulate), which often work in collaboration with mutant androgen receptors and are major determinants of derailed cellular activities.

It is appealing to note that Wnt5a triggers aggressiveness of prostate cancer and that its expression is involved in relapse after prostatectomy. Wnt5a activates Jun-N-terminal kinase through protein kinase D (PKD); furthermore, targeted inhibition of PKD suppresses Wnt5a-dependent cell migration and invasion. In addition,

Wnt5a stimulates expression of metalloproteinase-1 through recruitment of JunD to its promoter region. Similarly, suppression of Wnt 11 results in induction of apoptosis Fig. 2 (23,24).

It is appealing to note that Frizzled receptors are overexpressed in prostate cancer. However, captivatingly, this overexpression is counterbalanced by sFRP, which interacts with Frizzled receptors expressed in prostate cancer cells, and this heterodimer suppresses AR-mediated transactivation (25). There are various cell situations in which non-canonical Wnt signalling mediates cell-cell communication. Consistent with this notion, Wnt inhibitor Dickkopf-1 DKK-1 overexpression suppresses expression of p21(CIP1/WAF1) through a pathway independent of canonical Wnt signalling (26). However interestingly, keeping in view cancer promoting role of Dickkopf-1 (DKK-1), its appraisal as a potent inhibitor of bone growth in prostate cancer-induced osteoblastic metastases cannot be overlooked (27).

TNF-related apoptosis-inducing ligand (TRAIL) receptor agonistic agents and non-steroidal anti-inflammatory

Figure 2. Intricate crosstalk of various cytoplasmic proteins, which positively or negatively regulate signalling paradigms. Wnt attaches to Frizzled receptor and co-receptor LRP; however, it might also be inhibited by sFRP. β -catenin is degraded by machinery that is composed of Axin, GSK, CK and APC. It is also suppressed by caspase-8 and PTK6. Wnt signalling activates JNK via PKD. Osteoprotegerin competes with death receptors in offering binding sites for TRAIL and β -catenin translocates into the nucleus to switch on a variety of target genes.

drugs (NSAIDs) are attention-grabbing agents for chemoprevention and clinical management of cancer. In a set of experimentations, it has been observed that cells stably transfected with inducible dominant negative TCF-4 (dnTCF-4) construct serves to investigate the role of Wnt pathway activation. Both rhTRAIL-sensitive and -resistant cancer cell lines were robustly sensitized to rhTRAIL by aspirin. On the other hand, abrogation of TCF-4 completely blocked the sensitizing effect in cancer cells (28). Keeping in view the combination of TRAIL-receptor agonistic agents and NSAIDs as a striking treatment option for malignant tumours with constitutively active Wnt signalling, it is necessary to explore efficacy of the combinatorial approach in prostate cancer cell lines and models, as such models will provide vital information on the subject of efficacy of TRAIL-based therapies and potential toxicity in prostate cancer.

Concordantly, co-treatment of TRAIL-resistant cancer cells with TRAIL and the PPARGamma ligand troglitazone leads to suppression of β catenin expression, and enhancing rate of apoptosis. It is exciting to note that TRAIL- and troglitazone-induced apoptosis is preceded by cleavage of β catenin, mediated by caspases-3 and -8. Wide-ranging research is at present underway to discover therapeutic agents that can induce apoptosis distinctively in prostate cancer cells with minimal collateral damage to normal cells Fig. 2 (29).

It has recently been reported that tumour cells stimulate macrophages to release IL-1 β , which in turn opposes GSK3 β activity and simultaneously enhances Wnt signalling in cancer cells. This results in generation of a self-amplifying loop that promotes proliferation of tumour cells. Macrophages and IL-1 β have been shown to be unsuccessful in suppressing TRAIL-induced apoptosis in cells expressing dominant negative IkappaB, AKT or TCF4, confirming that they resist TRAIL-induced cell death through induction of the Wnt transduction cascade in tumour cells. To overcome this stumbling block, Vitamin D (3) has been described to be the interventional agent for this amplifying loop, by interfering with release of IL-1 β from macrophages (30). Accumulating data suggest that IL-1 β is a major participant in prostate cancer progression; it can activate the MAPK pathway and therefore ensues induction of IL-8, which escalates uncontrolled cell proliferation and invasive potential. Consistently along the same line, glucosamine inhibits IL-1 β -mediated activation of MAPKs and hence reduces IL-8 production (31).

The Wnt/ β catenin pathway contributes to carcinogenesis and cancer cell survival by driving expression of osteoprotegerin (OPG). OPG also serves as a decoy receptor for tumour necrosis factor-related apoptosis-inducing ligand (TRAIL). Expression of this survival factor, OPG,

might provide colorectal cancer cells with an essential growth advantage and contribute to cell invasion and metastasis Fig. 2 (32).

Accumulating evidence advocates co-culture of human pre-B leukaemia cells KM3 and REH with Wnt1- or Wnt3a-producing rat embryonic fibroblasts capably suppressed Apo2L/TRAIL-induced apoptosis of lymphoid cells (33). This highlights that Wnt1- or Wnt3a offer refractoriness in TRAIL-mediated therapy for prostate cancer. These proteins have to be explored more fully in prostate cancer. Similarly, GSK-3 β abrogation re-sensitizes prostate cancer cells to TRAIL-induced apoptosis (34).

Recently, research groups have documented zoledronic acid as an important therapeutic intervention in advanced PCa, as the WNT signalling pathway is upregulated and is regulated by zoledronic acid (35). Similarly, overexpression of fatty acid synthase (FASN) results in β -catenin protein accumulation and activation, whereas FASN abrogation by short-hairpin RNA results in suppression of β -catenin activation. It is thus, exciting to note that stabilization of β -catenin through palmitoylation of Wnt-1 and consequent activation of the pathway is a probable mechanism of FASN oncogenicity in prostate cancer progression (36). It has lately been explored that Mesd, a specialized chaperone for LRP5/LRP6, inhibits LRP6 phosphorylation and Wnt/ β catenin signalling in prostate cancer PC-3 cells, and inhibits PC-3 uncontrolled cell proliferation (37).

It is tempting to note that beta-TrCPs degrades caspase-3 by enhanced ubiquitination which severely dampens TRAIL-mediated efficacy because of suppression of caspase-3 (38). It has recently been documented that epigenetic deregulation of Wnt pathway inhibitors may contribute to aberrant activation of the Wnt signalling pathway, as there is quantitative increase in promoter methylation levels of APC associated with PCa progression (39,40).

Exceptional features of tumours that can be exploited by targeted therapies are a focal point of current cancer research. A detailed investigation into opportunities is necessary for realizing the full potentials of such therapies.

Wnt signalling-based prostate cancer models

It has recently been investigated in prostate cancer models in transgenic mice, induction of prostatic tumorigenesis, as well as tumour growth, was considerably enhanced by introduction of AR T877A mutation into prostate cells. Genetic screening of the mice revealed that Wnt-5a worked synchronously with mutant AR that resulted in transformation of cells. (41). On a similar note, prostates of mice expressing nuclear β -catenin alone developed prostate intraepithelial neoplasia (mPIN), whereas activa-

tion of the Wnt/ β -catenin pathway resulted in invasive prostate adenocarcinoma. Moreover, Foxa2, a forkhead transcription factor and matrix metalloproteinase, MMP7, were induced by active Wnt/ β -catenin signalling. It seems obvious that upregulated expression of Foxa2 and MMP7 was associated with the invasive phenotype in primary prostate cancer (42). Mounting data suggest that constitutive activation of this pathway induces castration-resistant prostate cancer in mouse. In accordance with the same concept, activation of β -catenin during early stages of prostate development has resulted in epithelial hyperplasia followed by prostatic intraepithelial neoplasia (PIN). Furthermore, in the adult prostate, activation of β -catenin gave rise to high-grade PIN (HGPIN) and uninterrupted prostatic growth after castration (43). Some drugs have been evaluated in mouse models with minimal side effects and tissue toxicity. This has the potential to be appreciated as a molecular medicine, as it has remarkable efficacy in mouse model having defect(s) in the adenomatous polyposis coli (APC)/ β -catenin regulatory system. It was also tested for tissue-specific efficacy in prostate cancer cell lines, DU145 and PC-3, in nude mouse xenografts and suppressed tumour growth in both (44).

Lately, it has also been explored that there is remarkable regression of tumour appearance in TRAIL-expressing mice compared to their wild-type littermates. More prominently, number of tumours observed in transgenic animals was considerably lower than in control animals, and also lesions observed were mostly benign. A further significant finding was differential expression of Wnt/ β -catenin signalling in tumours of wild-type and TRAIL transgenics (45).

A further important piece of information that improves our understanding is that deficiency in the adenomatous polyposis coli (APC) gene and consequent activation of β -catenin lead to suppression of cellular caspase-8 inhibitor c-FLIP (Fig. 2). Moreover, trans-retinyl acetate (RAc) independently upregulates tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) death receptors and suppresses expression of decoy receptors. Thus, a combinatorial drug design, particularly, TRAIL and RAc induces apoptosis in APC-deficient premalignant cells without affecting normal cells *in vitro* (46). It seems noticeable that APC is mutated in prostate cancer progression and that better understanding of the tumour microenvironment and cytosolic effectors of the Wnt signalling cascade will enable us to further approach translational oncology.

Therapeutic interventions for Wnt in prostate cancer

Curcumin suppresses β -catenin-mediated transcriptional activity (Teiten *et al.*, 2011) and soy-treated prostate

cancer PC3 cells display enhanced GSK3-mediated degradation of β -catenin (47). Analogously, multiple chemopreventive compounds (retinoid N-(4 hydroxyphe-nyl)retinamide (4HPR), Deguelin, a retinoid isolated from *Mundulea sericea*, indole-3-carbinol) trigger degradation of the β -catenin pathway (48–50).

It is very important to evaluate off-target effects of drugs at molecular levels. In support of this notion, ken-paullone (1, 3 μ M) and flutamide (10 μ M) enhance nuclear AR and β -catenin accumulation. Similarly, indirubin derivatives potentiate LNCaP cell proliferation (51). It is important to note that methyl jasmonate suppresses β -catenin mediated transcriptional activity and sensitizes cells to TRAIL (52).

A milestone in nutraceutical-based therapeutic interventions includes synthetic formulation and analogues of DIM and curcumin. Such formulation has remarkable efficacy in suppression of genomic rearrangements modulated by Wnt. Recent data suggest that BR-DIM and CDF suppress concomitant harmonized interaction of AR/TMPRSS2-ERG with Wnt (53). A different aspect is that enhanced catenin potentiates malignant transformation; however, targeted inhibition of β -catenin results in restoration of the normal cell phenotype of cell (54). Moreover, PKF118-310, suppresses Wnt/ β -catenin signalling and uncontrolled cell proliferation in prostate cancer cells (55).

Conclusion

Proteome underpinnings of prostate cancer are becoming progressively clearer owing to remarkable and well-coordinated ventures occurring worldwide. Over-expression of negative regulators of apoptosis compromise cell functions and can reduce cell fitness or contribute to malignant transformation. As a counter-measure, normal cells have developed strategies for eliminating their cancerous neighbours. Prostate carcinogenesis is driven by a complex signalling cascade, but, from a functional perspective, it can be defined as an oncoprogressive mechanism. As our understanding of molecular alterations driving prostate cancer has increased outstandingly, there is the chance to redirect clinical application of prostate cancer therapeutics with enhanced accuracy. Accordingly, it is fundamental that full-bodied translational strategies be developed pre-clinically to both lessen failure rates in the clinic and to curtail time required to identify patient populations most probable to benefit from a certain therapy. It is getting increasingly significant to evaluate pre-clinical model systems, we being conscious of the fact that multipronged approaches will be needed.

References

- Vondálová Blánárová O, Jelínková I, Szöör A, Skender B, Soucek K, Horváth V *et al.* (2011) Cisplatin and a potent platinum(IV) complex-mediated enhancement of TRAIL-induced cancer cells killing is associated with modulation of upstream events in the extrinsic apoptotic pathway. *Carcinogenesis* **32**, 42–51.
- Brauer PM, Zheng Y, Evans MD, Dominguez-Brauer C, Peehl DM, Tyner AL (2011) The alternative splice variant of protein tyrosine kinase 6 negatively regulates growth and enhances PTK6-mediated inhibition of β -catenin. *PLoS ONE* **6**, e14789.
- Wu XX, Kakehi Y (2009) Enhancement of lexatumumab-induced apoptosis in human solid cancer cells by Cisplatin in caspase-dependent manner. *Clin. Cancer Res.* **15**, 2039–2047.
- Shimada O, Wu X, Jin X, Nouh MA, Fiscella M, Albert V *et al.* (2007) Human agonistic antibody to tumor necrosis factor-related apoptosis-inducing ligand receptor 2 induces cytotoxicity and apoptosis in prostate cancer and bladder cancer cells. *Urology* **69**, 395–401.
- Yu M, Gipp J, Yoon JW, Iannaccone P, Walterhouse D, Bushman W (2009) Sonic hedgehog-responsive genes in the fetal prostate. *J. Biol. Chem.* **284**, 5620–5629.
- Blum R, Gupta R, Burger PE, Ontiveros CS, Salm SN, Xiong X *et al.* (2009) Molecular signatures of prostate stem cells reveal novel signaling pathways and provide insights into prostate cancer. *PLoS ONE* **4**, e5722.
- Mimeault M, Johansson SL, Vankatraman G, Moore E, Henichart JP, Depreux P *et al.* (2007) Combined targeting of epidermal growth factor receptor and hedgehog signaling by gefitinib and cyclopamine cooperatively improves the cytotoxic effects of docetaxel on metastatic prostate cancer cells. *Mol. Cancer Ther.* **6**, 967–978.
- Tzelepi V, Karlou M, Wen S, Hoang A, Logothetis C, Troncoso P *et al.* (2011) Expression of hedgehog pathway components in prostate carcinoma microenvironment: shifting the balance towards autocrine signalling. *Histopathology* **58**, 1037–1047.
- Shigemura K, Huang WC, Li X, Zhou HE, Zhu G, Gotoh A *et al.* (2011) Active sonic hedgehog signaling between androgen independent human prostate cancer cells and normal/benign but not cancer-associated prostate stromal cells. *prostate*. doi: 10.1002/pros.21388.
- Chang HH, Chen BY, Wu CY, Tsao ZJ, Chen YY, Chang CP *et al.* (2011) Hedgehog overexpression leads to the formation of prostate cancer stem cells with metastatic property irrespective of androgen receptor expression in the mouse model. *J. Biomed. Sci.* **18**, 6.
- Chen G, Goto Y, Sakamoto R, Tanaka K, Matsubara E, Nakamura M *et al.* (2011) GLI1, a crucial mediator of sonic hedgehog signaling in prostate cancer, functions as a negative modulator for androgen receptor. *Biochem. Biophys. Res. Commun.* **404**, 809–815.
- Azoulay S, Terry S, Chimingqi M, Sirab N, Faucon H, Gil Diez-deMedina S *et al.* (2008) Comparative expression of Hedgehog ligands at different stages of prostate carcinoma progression. *J. Pathol.* **216**, 460–470.
- Shaw A, Gipp J, Bushman W (2009) The Sonic Hedgehog pathway stimulates prostate tumor growth by paracrine signaling and recapitulates embryonic gene expression in tumor myofibroblasts. *Oncogene* **28**, 4480–4490.
- Chung MK, Kim HJ, Lee YS, Han ME, Yoon S, Baek SY *et al.* (2010) Hedgehog signaling regulates proliferation of prostate cancer cells via stathmin1. *Clin. Exp. Med.* **10**, 51–57.
- Shaw A, Gipp J, Bushman W (2010) Exploration of Shh and BMP paracrine signaling in a prostate cancer xenograft. *Differentiation* **79**, 41–47.
- Leow CC, Wang BE, Ross J, Chan SM, Zha J, Carano RA *et al.* (2009) Prostate-specific Klf6 inactivation impairs anterior prostate branching morphogenesis through increased activation of the Shh pathway. *J. Biol. Chem.* **284**, 21057–21065.
- Zunich SM, Douglas T, Valdovinos M, Chang T, Bushman W, Walterhouse D *et al.* (2009) Paracrine sonic hedgehog signalling by prostate cancer cells induces osteoblast differentiation. *Mol. Cancer* **8**, 12.
- Kasperczyk H, Baumann B, Debatin KM, Fulda S (2009) Characterization of sonic hedgehog as a novel NF-kappaB target gene that promotes NF-kappaB-mediated apoptosis resistance and tumor growth in vivo. *FASEB J.* **23**, 21–33.
- Kump E, Ji J, Wernli M, Häusermann P, Erb P (2008) Gli2 upregulates cFlip and renders basal cell carcinoma cells resistant to death ligand-mediated apoptosis. *Oncogene* **27**, 3856–3864.
- Kurita S, Mott JL, Almada LL, Bronk SF, Werneburg NW, Sun SY *et al.* (2010) GLI3-dependent repression of DR4 mediates hedgehog antagonism of TRAIL-induced apoptosis. *Oncogene* **29**, 4848–4858.
- Büttner A, Seifert K, Cottin T, Sarli V, Tzagkaroulaki L, Scholz S *et al.* (2009) Synthesis and biological evaluation of SANT-2 and analogues as inhibitors of the hedgehog signaling pathway. *Bioorg. Med. Chem.* **17**, 4943–4954.
- Huang SP, Ting WC, Chen LM, Huang LC, Liu CC, Chen CW *et al.* (2010) Association analysis of Wnt pathway genes on prostate-specific antigen recurrence after radical prostatectomy. *Ann. Surg. Oncol.* **17**, 312–322.
- Yamamoto H, Oue N, Sato A, Hasegawa Y, Yamamoto H, Matsubara A *et al.* (2010) Wnt5a signaling is involved in the aggressiveness of prostate cancer and expression of metalloproteinase. *Oncogene* **29**, 2036–2046.
- Uysal-Onganer P, Kawano Y, Caro M, Walker MM, Diez S, Darrington RS *et al.* (2010) Wnt-11 promotes neuroendocrine-like differentiation, survival and migration of prostate cancer cells. *Mol. Cancer* **9**, 55.
- Kawano Y, Diez S, Uysal-Onganer P, Darrington RS, Waxman J, Kypta RM (2009) Secreted Frizzled-related protein-1 is a negative regulator of androgen receptor activity in prostate cancer. *Br. J. Cancer* **100**, 1165–1174.
- Hall CL, Zhang H, Baile S, Ljungman M, Kuhstoss S, Keller ET (2010) p21CIP-1/WAF-1 induction is required to inhibit prostate cancer growth elicited by deficient expression of the Wnt inhibitor Dickkopf-1. *Cancer Res.* **70**, 9916–9926.
- Thudi NK, Martin CK, Murahari S, Shu ST, Lanigan LG, Werbeck JL *et al.* (2011) Dickkopf-1 (DKK-1) stimulated prostate cancer growth and metastasis and inhibited bone formation in osteoblastic bone metastases. *Prostate* **71**, 615–625.
- Heijink DM, Jalving M, Oosterhuis D, Sloots IA, Koster R, Hollema H *et al.* (2011) TNF-related apoptosis-inducing ligand cooperates with NSAIDs via activated Wnt signalling in (pre)malignant colon cells. *J. Pathol.* **223**, 378–389.
- Senthivayagam S, Mishra P, Paramasivam SK, Yallapragada S, Chatterjee M, Wong L *et al.* (2009) Caspase-mediated cleavage of β catenin precedes drug-induced apoptosis in resistant cancer cells. *J. Biol. Chem.* **284**, 13577–13588.
- Kaler P, Galea V, Augenlicht L, Klampfer L (2010) Tumor associated macrophages protect colon cancer cells from TRAIL-induced apoptosis through IL-1 β -dependent stabilization of Snail in tumor cells. *PLoS ONE* **5**, e11700.
- Tsai CY, Lee TS, Kou YR, Wu YL (2009) Glucosamine inhibits IL-1 β -mediated IL-8 production in prostate cancer cells by MAPK attenuation. *J. Cell. Biochem.* **108**, 489–498.
- De Toni EN, Thieme SE, Herbst A, Behrens A, Stieber P, Jung A *et al.* (2008) OPG is regulated by β catenin and mediates resistance

- to TRAIL-induced apoptosis in colon cancer. *Clin. Cancer Res.* **14**, 4713–4718.
- 33 Doubravská L, Símová S, Cermak L, Valenta T, Korínek V, Andera L (2008) Wnt-expressing rat embryonic fibroblasts suppress Apo2L/TRAIL-induced apoptosis of human leukemia cells. *Apoptosis* **13**, 573–587.
 - 34 Liao X, Zhang L, Thrasher JB, Du J, Li B (2003) Glycogen synthase kinase-3 β suppression eliminates tumor necrosis factor-related apoptosis-inducing ligand resistance in prostate cancer. *Mol. Cancer Ther.* **2**, 1215–1222.
 - 35 Thiele S, Rauner M, Goettsch C, Rachner TD, Benad P, Fuessel S *et al.* (2011) Expression profile of WNT molecules in prostate cancer and its regulation by aminobisphosphonates. *J. Cell. Biochem.* **112**, 1593–1600.
 - 36 Fiorentino M, Zadra G, Palescandolo E, Fedele G, Bailey D, Fiore C *et al.* (2008) Overexpression of fatty acid synthase is associated with palmitoylation of Wnt1 and cytoplasmic stabilization of β catenin in prostate cancer. *Lab. Invest.* **88**, 1340–1348.
 - 37 Lu W, Liu CC, Thottassery JV, Bu G, Li Y (2010) Mesd is a universal inhibitor of Wnt coreceptors LRP5 and LRP6 and blocks Wnt/ β catenin signaling in cancer cells. *Biochemistry* **49**, 4635–4643.
 - 38 Tan M, Gallegos JR, Gu Q, Huang Y, Li J, Jin Y *et al.* (2006) SAG/ROC-SCF β -TrCP E3 ubiquitin ligase promotes pro-caspase-3 degradation as a mechanism of apoptosis protection. *Neoplasia* **8**, 1042–1054.
 - 39 Liu L, Kron KJ, Pethe VV, Demetrashvili N, Nesbitt ME, Trachtenberg J *et al.* (2011) Association of tissue promoter methylation levels of APC, TGF β 2, HOXD3, and RASSF1A with prostate cancer progression. *Int. J. Cancer AOP* doi: 10.1002/ijc.25908.
 - 40 Costa VL, Henrique R, Ribeiro FR, Carvalho JR, Oliveira J, Lobo F *et al.* (2010) Epigenetic regulation of Wnt signaling pathway in urological cancer. *Epigenetics* **5**, 343–351.
 - 41 Takahashi S, Watanabe T, Okada M, Inoue K, Ueda T, Takada I *et al.* (2011) Noncanonical Wnt signaling mediates androgen-dependent tumor growth in a mouse model of prostate cancer. *Proc. Natl. Acad. Sci. USA* **108**, 4938–4943.
 - 42 Yu X, Wang Y, DeGraff DJ, Wills ML, Matusik RJ (2011) Wnt/ β -catenin activation promotes prostate tumor progression in a mouse model. *Oncogene* **30**, 1868–1879.
 - 43 Yu X, Wang Y, Jiang M, Brier B, Roy-Burman P, Shen MM *et al.* (2009) Activation of β catenin in mouse prostate causes HGPIN and continuous prostate growth after castration. *Prostate* **69**, 249–262.
 - 44 Symolon H, Bushnev A, Peng Q, Ramaraju H, Mays SG, Allegood JC *et al.* (2011) Enigmol: a novel sphingolipid analogue with anticancer activity against cancer cell lines and in vivo models for intestinal and prostate cancer. *Mol. Cancer Ther.* **10**, 648–657.
 - 45 Kedinger V, Muller S, Gronemeyer H (2011) Targeted expression of tumor necrosis factor-related apoptosis-inducing ligand TRAIL in skin protects mice against chemical carcinogenesis. *Mol. Cancer* **10**, 34.
 - 46 Zhang L, Ren X, Alt E, Bai X, Huang S, Xu Z *et al.* (2010) Chemoprevention of colorectal cancer by targeting APC-deficient cells for apoptosis. *Nature* **464**, 1058–1061.
 - 47 Liss MA, Schlicht M, Kahler A, Fitzgerald R, Thomassi T, Degueme A *et al.* (2010) Characterization of soy-based changes in Wnt-frizzled signaling in prostate cancer. *Cancer Genomics Proteomics* **7**, 245–252.
 - 48 Benelli R, Monteghirfo S, Venè R, Tosetti F, Ferrari N (2010) The chemopreventive retinoid 4HPR impairs prostate cancer cell migration and invasion by interfering with FAK/AKT/GSK3 β pathway and β catenin stability. *Mol. Cancer* **9**, 142.
 - 49 Thamilselvan V, Menon M, Thamilselvan S (2011) Anticancer efficacy of deguelin in human prostate cancer cells targeting glycogen synthase kinase-3 β / β -catenin pathway. *Int. J. Cancer*. doi: 10.1002/ijc.25949.
 - 50 Jeong YM, Li H, Kim SY, Yun HY, Baek KJ, Kwon NS *et al.* (2011) Indole-3-carbinol inhibits prostate cancer cell migration via degradation of β catenin. *Oncol. Res.* **19**, 237–243.
 - 51 Rivest P, Renaud M, Sanderson JT (2011) Proliferative and androgenic effects of indirubin derivatives in LNCaP human prostate cancer cells at sub-apoptotic concentrations. *Chem. Biol. Interact.* **189**, 177–185.
 - 52 Raviv Z, Zilberberg A, Cohen S, Reischer-Pelech D, Horrix C, Berger MR *et al.* (2011) Methyl jasmonate downregulates survivin expression and sensitizes colon carcinoma cells towards TRAIL-induced cytotoxicity. *Br. J. Pharmacol.*, doi: 10.1111/j.1476-5381.2011.01419.x.
 - 53 Li Y, Kong D, Wang Z, Ahmad A, Bao B, Padhye S *et al.* (2011) Inactivation of AR/TMPRSS2-ERG/Wnt signaling networks attenuates the aggressive behavior of prostate cancer cells. *Cancer Prev. Res. (Phila) AOP* **4**, 1495–1506.
 - 54 Zhao JH, Luo Y, Jiang YG, He DL, Wu CT (2011) Knockdown of β -catenin through shRNA cause a reversal of EMT and metastatic phenotypes induced by HIF-1 α . *Cancer Invest.* **29**, 377–382.
 - 55 Lu W, Tinsley HN, Keeton A, Qu Z, Piazza GA, Li Y (2009) Suppression of Wnt/ β catenin signaling inhibits prostate cancer cell proliferation. *Eur. J. Pharmacol.* **602**, 8–14.