The molecular basis of prostate cancer

Cancer involves accumulation of genetic alterations. This review highlights the alterations in control pathways for cell division, development, DNA repair, angiogenesis and cell death that are believed to be key players in the development of prostate cancer.

Over the past two decades considerable progress has been made in unravelling the molecular mechanisms of cancer development. It is now generally accepted that cancer involves the accumulation of genetic mutations in three classes of genes that are responsible for tumour development: oncogenes that drive cell growth; suppressor genes whose removal allows cells to grow out of control; and stability genes that, when altered, allow cells to more rapidly accumulate genetic alterations in oncogenes and suppressor genes. Mutations in several of these genes together in a single cell are usually required for the development of a malignant cancer (Vogelstein and Kinzler, 2004). At the DNA level oncogenes may be activated by point mutations, chromosomal translocations or gene amplifications, while removal of suppressor genes can result from mutations that inactivate or truncate the encoded protein or from deletions within or encompassing the gene. When alterations in any of these three classes of genes are present in the germ line, inherited predispositions to cancer may result.

This article will review the current state of knowledge on the molecular genetic mechanisms of development of prostate cancer. What has emerged in the study of other cancer types is that the genetic alterations usually act by deregulating particular control pathways, for example, pathways that control cell division, cell death, angiogenesis and protein stability (Vogelstein and Kinzler, 2004). This review will therefore focus specifically on the alteration in control pathways believed to be important in modulating the growth of human prostate cancer.

Glossary

APC = adenomatous polyposis of the colon
BAD = BCL2 antagonist of cell death
Bcl-2= B-cell CLL/lymphoma 2
BRCA2 = breast cancer 2 gene
E2F3 = E2F transcription factor 3
FOXO1A = Forkhead box O1A
GLI = glioma-associated oncogene homolog
GSK3 = glycogen synthase kinase 3
LEF = lymphoid enhancer-binding factor
PI3K = phosphoinositide 3-kinase
PIP3 = phosphatidylinositol-3-phosphate
PKB = protein kinase B
pRB = retinoblastoma protein
PTEN = phosphatase and tensin homolog
PTK = protein tyrosine kinase
TCF = transcription factor
TRAMP = transgenic adenocarcinoma mouse prostate
TSC2= tuberous sclerosis 2
WNT = wingless-type mmtv integration site family

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The androgen receptor pathway

In the normal prostate androgens act through a paracrine mechanism: activation of the androgen receptor (AR) in stromal cells results in secretion of peptide growth factors that in turn control proliferation and survival of adjacent prostate epithelial cells. Within the normal prostate epithelial cells, the AR also controls transcription of a series of highly expressed genes, such as prostate-specific antigen (PSA), but acts to suppress growth rather than to stimulate it. In prostate cancers, major changes in this control circuitry are found such that the growth of prostate cancer epithelial cells becomes directly dependent on signalling through the AR. Accordingly the mainstay of treatment for prostate cancer that has spread outside the prostate gland involves withdrawal of the androgens that are required for the cancer growth. Almost invariably, however, the cancer eventually becomes refractory to this treatment (hormone refractory, HR) and the cancer cells regrow even in the presence of very low androgen levels.

Several lines of evidence indicate that alteration of the AR itself can play a key role in development of HR cancer. Chen et al (2004) provided evidence that low level (2–5-fold) elevation in AR transcripts was both necessary and sufficient to induce hormone resistance in animal model systems, apparently mimicking human prostate cancer where elevated AR levels are observed in around 40% of patients with HR disease. In addition, mutations and amplifications of the AR gene are found in a small proportion of HR cancer. Alteration in the AR, however, is not the only mechanism of development of HR cancer and other models must be considered, including alteration of other components of the AR pathway (e.g. co-repressor and co-activators), completely androgen-independent routes and modulation of AR by other control pathways. For example, Mellinghoff et al (2004) have shown that signalling through the HER2/ERBB2 tyrosine kinase receptor pathway can stabilize AR protein levels and optimize binding of AR to promoter/enhancer regions of androgen response genes.

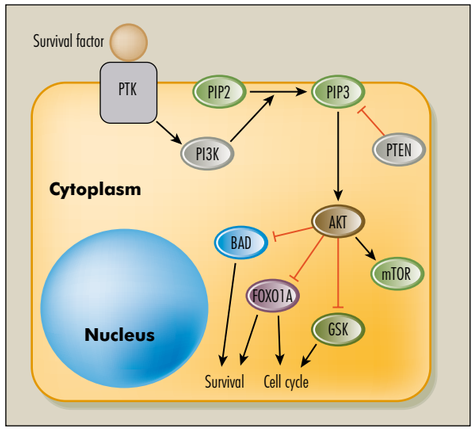


Figure 1. Activated protein tyrosine kinase (PTK) receptors recruit phosphoinisitide 3-kinase (P13K) to the cell membrane; this stimulates the formation of phosphatidylinositol-3-phosphate (PIP3). PIP3 activates AKT that in turn affects several number pathways, e.g. FOXO1A (cell cycle), BAD (cell survival), glycogen synthase kinase 3 (GSK3) (cell cycle) and the mTOR pathways (nutrient response). PIP3 can be removed by phosphatase and tensin homolog (PTEN).

PI3K/AKT pathway

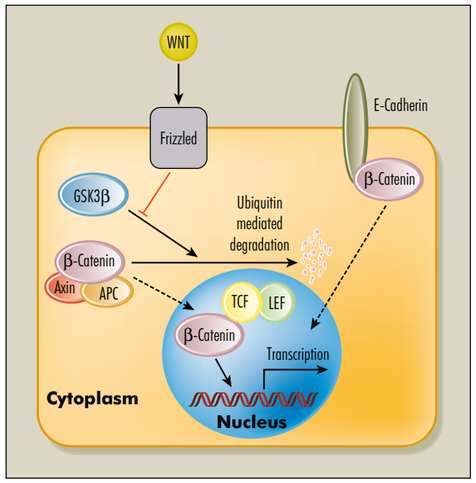
Signalling through the PI3K/AKT pathway links cell growth and survival signals received by cell surface receptor tyrosine kinase receptors to internal cellular control mechanisms (*Figure 1*). Activation of PTK receptors results in recruitment of PI3K to the cell membrane and generation of the lipid PIP3. PIP3 in turn recruits PKB/AKT to the plasma membrane resulting in AKT activation and, through phosphorylation, modulates a number of downstream pathways including FOXO1A (cell cycle), BAD (cell survival), GSK3 (cell cycle) and the TSC2/mTOR pathways (nutrient response). A protein called PTEN, a lipid phosphatase, downregulates this pathway by removing the critical phospholipid PIP3 (*Figure 1*).

Several lines of evidence support the view that loss of PTEN is important in prostate cancer development (reviewed in Sansal and Sellers, 2004). Loss of a single copy (loss of heterozygosity; LOH) of chromosome 10q23, the location of PTEN, is found in up to 20% of primary cancers and 60% of metastatic cancers. Furthermore, in primary cancer the loss of this locus is correlated with high tumour grade and stage. Mutations in the remaining PTEN allele have been reported in prostate cancer cell lines and xenographs and in up to 50% of metastatic cancers. The reported incidence of such mutations varies widely, however, and the mutations are generally much less common than the LOH at 10q23. The development of prostate cancer in animal models (e.g. the PTEN+/- TRAMP mouse) that contain heterozygous deletions of one PTEN allele also supports the importance of this gene

The WNT pathway

In the absence of signalling through the WNT pathway, β-catenin is present at low levels in cells as a result of its constitutive degradation by proteasomes (*Figure 2*). This degradation is controlled by APC and Axin, which are responsible for presenting β-catenin for phosphorylation at its N-terminus by GSK3β. Signalling induced by WNT, which acts by binding to the Frizzled receptor, inhibits GSK3β resulting in β-catenin stabilization. β-catenin then accumulates in the nucleus and interacts with the TCF/LEF transcription factors thus modulating the expression of specific genes. The cellular location of β-catenin is influenced by E-cadherin, the cytoplasmic domain of which can bind to β-catenin holding it at the cell surface. It has been established that phosphorylation of GSK3β by AKT can also contribute to the

Figure 2. β-catenin is constitutively degraded by proteasomes. This degradation is dependent on phosphorlyation by glycogen synthase kinase 3β (GSK3β) and requires the presence of APC and Axin. Signalling induced by WNT binding to the Frizzled receptor, inhibits GSK3β resulting in β-catenin stabilization. β-catenin then accumulates in the nucleus and interacts with the TCF/LEF transcription factors in modulating the expression of specific genes. The binding to E-cadherin can also prevent accumulation of β-catenin in the nucleus.



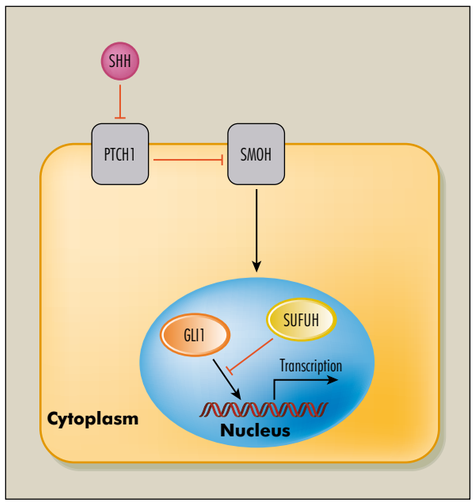
downregulation of GSK3β and to β-catenin activation (reviewed in Verras and Sun, 2005).

Analysis of prostate cancer has demonstrated accumulation of nuclear β-catenin in around 25% of cases. In a fifth of these cases (5% of the total) the accumulation of β-catenin is a result of mutations in the β-catenin gene that prevent phosphorylation and hence degradation of the encoded protein. It is possible that in the remaining cases β-catenin accumulation may result from other mechanisms, such as E-cadherin downregulation or abnormal expression of WNT ligands, WNT receptors or co-factors. Interestingly, β-catenin can also bind directly to AR causing its nuclear localization in an APC and GSK3β independent fashion.

The Sonic Hedgehog–GLI1 pathway

Sonic Hedgehog (SHH) signalling is essential for the formation of external male genitalia and the prostate. The SHH–GLI1 pathway is highly controlled and it is normally only activated at precise time points and locations during development. In prostate cancer this pathway appears to be activated. Prostate cancers consistently express GLI1 and there is clear upregulation of both Patched1 (PTCH1) and GLI1 in metastatic prostate cancer compared to benign tumours and normal tissue. Furthermore, Suppression of fused H (SUFUH) is expressed in human prostate cancers with low Gleason score but not in those with high Gleason scores. In support of the importance of this pathway, in vitro removal of GLI1 reduces proliferation rates drastically (Sanchez et al, 2004).

Figure 3. Sonic Hedgehog (SHH) inhibits the repression of Smoothened (SMOH) by Patched1 (PTCH1). SMOH activates glioma-associated oncogene homolog (GLI1) that regulates transcription of specific genes. This can, however, be inhibited by Suppressor of fused (SUFUH).



Cyclopamine (an inhibitor of Smoothened (SMOH), *Figure 3*) is also an effective inhibitor of cell proliferation of prostate cancer cells in vitro and, as anticipated, this inhibitory effect can be bypassed by GLI1 overexpression. SHH expression has also been shown to induce angiogenesis and facilitate the growth of LNCaP and PC3 prostate cancer cells in mouse xenografts. Further, SMOH and SUFUH have been found to be located in chromosomal regions implicated in familial prostate cancer. These studies indicate that activation of the SHH–GLI1 pathway is essential for the development of prostate cancer and has a high potential as a therapeutic target (Sanchez et al, 2005).

The pRB–E2F axis

Mutations of cell cycle-regulating factors are frequent events in cancer cells. At least one of the following alterations is found in a majority of cancers: overexpression of cyclins, activation of cyclin-dependent kinases (CDK), inactivation of CDK inhibitors and/or loss of pRB expression. These events all result in inactivation of the pRB tumour suppressor function, and they result in growth advantage and allow bypass of cellular senescence pathways (Malumbres and Barbacid, 2001). Loss of heterozygosity of pRB is seen in 17–60% of all prostate cancers, and is believed to be an early event in tumour development. Deletion of one RB allele in mouse prostate gives rise to focal prostate hyperplasia, however, these lesions do not become malignant, indicating that further genetic alterations are needed to promote malignant transformation (Maddison et al, 2004).

pRB plays a critical role in cell cycle progression by binding and inhibiting E2Fs, and hence preventing the S phase entry, until it is phosphorylated by CDKs. High nuclear expression of E2F3 has been identified in 67% of human prostate cancer although this high level expression has not been linked to DNA amplification of the E2F3 encoding gene. It has been hypothesized that RB1 inactivation and E2F3 overexpression cooperates in human prostate cancer. Upregulation of E2F3 is strongly correlated with stage and grade of prostate cancer and may serve as an independent marker for poor clinical outcome (Foster et al, 2004).

Histone modifiers

Polycomb proteins are responsible for linking histone modifications such as acelylation and modification to transcriptional repression. The polycomb protein Enhancer of Zeste 2 (EZH2) is overexpressed in metastatic and HR prostate cancer, occasionally associated with DNA amplification, and can be used as a marker for prostate cancer aggressiveness (Varambally et al, 2002). In accordance with this view, downregulation of EZH2 in prostate cancer cell lines inhibits proliferation in vitro. Consistent with the importance of histone modification Seligson et al (2005) performed immunohistochemistry on tissue micro array on 183 primary prostate cancers and showed that changes in histone acetylation and demethylation of five residues in histones H3 and H4 in patients with low-grade prostate cancer, were predictive of clinical outcome.

Another histone modifier that shows altered activity in cancer is p300 histone acetyl transferase. p300 is involved in prostate cancer proliferation and levels of this protein correlate with disease progression. Furthermore, p300 appears to be involved in AR co-activation and could be a factor in the progression towards androgen independence (Debes et al, 2003).

Mitosis

During mitosis, the duplicated genomes are segregated into two daughter cells. This process requires condensation of chromosomes, centrosomes organizing the microtubule network during interphase, formation of a perfect mitotic spindle during cell division, and cytokineses. These events are controlled through phosphorylation by mitotic kinases, e.g. Polo-like kinases (Plk), CDK1 and Aurora-related kinases. Failure at any of these events is tightly correlated with aneuploidy and chromosome instability that can lead to tumour development.

Aurora A is an oncogene located on human 20q13, a DNA locus that is frequently gained in prostate cancer. Overexpression of this gene has been reported in numerous cancer types including prostate cancer. Upregulation of Aurora A results in abnormalities in G2 and spindle checkpoints and cytokinesis failure in vitro. Inhibition of aurora-kinases effectively blocks cell growth and induces apoptosis in cancer cells. Aurora B phosphorylates Survivin which is correlated with proliferation, stage and grade of prostate cancer (Kishi et al, 2004). Plk1 is almost undetectable in normal prostate cells, but highly upregulated in prostate cancer cells as in most other human tumours. Plk1 depletion results in a 45–72% decreased cell viability, as a result of induction of apoptosis, and reduced cell growth in prostate cancer cells, while normal prostate epithelial cells are unaffected in vitro (Reagan-Shaw and Ahmad, 2005). Plk1 is involved in the spindle formation and chromosome segregation during mitosis and deregulation of Plk1 seems to be an early event in oncogenic transformation of human cells. Plk1 seems a very promising therapeutic target in prostate cancer.

DNA repair pathways

Mutations in a variety of genes involved in controlling DNA stability (e.g. MSH2, MLH1, XPA, ATM) have been implicated in cancer development. Mutations of these genes have occasionally been reported in prostate cancer and their alterations are more commonly associated with development of other cancer types. The breast cancer predisposition gene BRCA2, involved in double strand DNA break repair, has recently been implicated in the development of human prostate cancer: men harbouring inherited mutations in the BRCA2 gene are at higher risk of developing prostate cancer and interestingly, the spectrum of mutation responsible for familial predisposition to prostate cancer is distinct from that usually observed in breast cancer families (Farmer et al, 2005).

It has recently been shown that cells defective in the BRCA2 gene are profoundly sensitized to inhibition of Poly (ADP-ribose) polymerase (PARP), an enzyme involved in base excision repair. This sensitization occurs because inhibition of PARP leads to the generation of DNA double strand breaks that would normally be repaired by BRCA2-mediated homologous recombination. In the absence of both pathways, the cells cannot repair double strand breaks and die. This observation raised the intriguing possibility that PARP inhibitors may be of use in the treatment of prostate cancer arising in patients with inherited BRCA2 mutations or in spontaneous cases of this disease that have arisen through inactivation of BRCA2.

Apoptosis

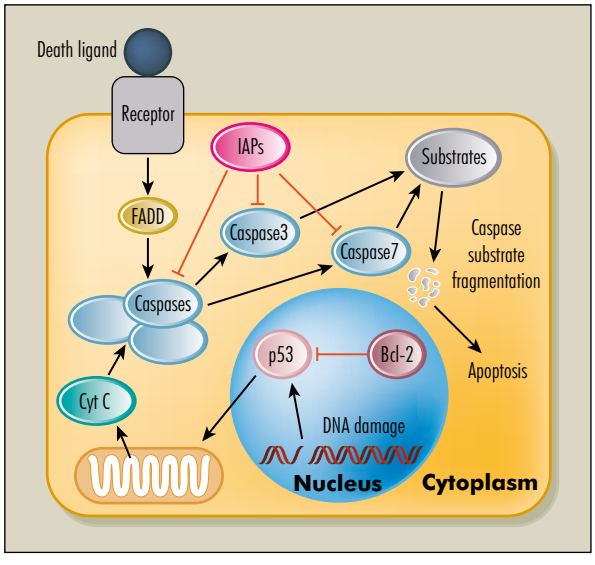
Apoptosis is an essential event in normal mammalian development both during embryogenesis and maintenance of tissue homeostasis. However, failure to undergo normal apoptosis can contribute to cancer development. Most epithelial and endothelial cells undergo apoptosis when they lose contact with the extracellular matrix, a phenomenon referred to as anoikis. Increasing evidence indicates that impaired ability to undergo apoptosis plays an important role in development of primary prostate cancer and, in progression from androgen-dependent to androgen-independent prostate cancer.

Survivin, a member of the Inhibitors of Apoptosis (IAPs) family, is involved in chromosome congression, spindle microtubule function and mitochondrial-dependent apoptosis. Survivin is not normally expressed in the prostate epithelial cells, but is strongly expressed in prostate cancer cells and the level of expression is correlated with progression and aggressiveness of prostate cancer. In-vitro removal of Survivin inhibits cell proliferation by disrupting the chromosome orientation, and changes the levels of Caspase-3 and -7, the terminal effectors of apoptosis (*Figure 4*) (Kishi et al, 2004). Survivin has also been implicated in hormone therapy resistance, and there are indications that targeting Survivin may enhance sensitivity to anti-androgen therapy in prostate cancer (Zhang et al, 2005). XIAP, another member of this family, has been found to be upregulated in prostate cancer and may contribute to anoikis resistance. Initial preclinical data also suggests that a small molecular antagonist of XIAP possesses antimetastatic activity (Berezovskaya et al, 2005).

The p53 gene is located in a region on chromosome 17 that is commonly deleted in advanced stages of prostate cancer and metastatic disease (Cher et al, 1996). The remaining p53 allele frequently harbours

point mutations, an event that is associated with poor prognosis. Apoptosis induced by different types of molecular stress through p53 can be inhibited by Bcl-2, a gene that is overexpressed in 30% of prostate cancers and whose expression is correlated with poor clinical outcome. Knockout of Bcl-2 mRNA can significantly enhance the therapeutic effect of chemotherapy, hormone and radiation therapy in patients with HR prostate cancer. Apoptosis as a genetically regulated process has an endpoint that coincides with the therapeutic goal of successful treatment, and the idea of altering the apoptotic threshold of prostate cancer cells is very appealing.

Figure 4. Programmed cell death (apoptosis) can be triggered by different stimuli binding of death ligands to their receptors triggers a proteolytic cascade of caspases; Caspase-3 and -7 are the effectors that degrade the caspase substrates which leads to apoptosis. Apoptosis can also be the end result of DNA damage via p53 and cytochrome C activation of the caspases: this pathway can be inhibited by Bcl-2. The only known inhibitors of the caspases are the members of the Inhibitors of Apoptosis (IAPs) family.



KEY POINTS

- Loss of PTEN disturbs the PI3K/AKT pathway and is an important step in prostate cancer development as well as correlated with tumour grade and stage
- Activation of the SHH–GLI1 pathway is essential for the development of prostate cancer and affects the proliferation rate, progression and tumour invasiveness.
- RB1 inactivation and E2F3 overexpression is believed to cooperate in prostate cancer and the latter can serve as a negative marker of survival.
- Plk depletion results in drastic decrease of cell viability and growth rate of prostate cancer cells, but leave normal prostate cells unaffected.
- Prostate cancer cells harbouring BRCA2 mutations that are exposed to PARP inhibitors cannot perform DNA repair and dies.
- Survivin expression is strongly correlated with progression and aggression of prostate cancer and in vitro removal inhibit cell proliferation.

Conclusions

Several key cellular control pathways have been implicated in the development of human prostate cancer. In many of these cases, however, although alterations in the protein levels of pathway components have been established, the underlying alterations in genes encoding the proteins have yet to be identified. Nonetheless, this information allows the rational design of drugs that can target important controlling pathways and that may be of potential benefit to prostate cancer patients. **BJHM**

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Berezovskaya O, Schimmer AD, Glinskii AB et al (2005) Increased expression of apoptosis inhibitor protein XIAP contributes to anoikis resistance of circulating human prostate cancer metastasis precursor cells. *Cancer Res* **65:** 2378–86

Chen CD, Welsbie DS, Tran C et al (2004) Molecular determinants of resistance to antiandrogen therapy. *Nat Med* **10:** 33–9

Cher ML, Bova GS, Moore DH et al (1996) Genetic alterations in untreated metastases and androgen-independent prostate cancer detected by comparative genomic hybridization and allelotyping. *Cancer Res* **56:** 3091–102

Debes JD, Sebo TJ, Lohse CM, Murphy LM, Haugen DA, Tindall DJ (2003) p300 in prostate cancer proliferation and progression. *Cancer Res* **63:** 7638–40

Farmer H, McCabe N, Lord CJ et al (2005) Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature* **434:** 917–21

Foster CS, Falconer A, Dodson AR et al (2004) Transcription factor E2F3 overexpressed in prostate cancer independently predicts clinical outcome. *Oncogene* **23:** 5871–979

Kishi H, Igawa M, Kikuno N, Yoshino T, Urakami S, Shiina H (2004) Expression of the survivin gene in prostate cancer: correlation with clinicopathological characteristics, proliferative activity and apoptosis. *J Urol* **171:** 1855–60

Maddison LA, Sutherland BW, Barrios RJ, Greenberg NM (2004) Conditional deletion of Rb causes early stage prostate cancer. *Cancer Res* **64:** 6018–25

Malumbres M, Barbacid M (2001) To cycle or not to cycle: a critical decision in cancer. *Nat Rev Cancer* **1:** 222–31

Mellinghoff IK, Vivanco I, Kwon A, Tran C, Wongvipat J, Sawyers CL (2004) HER2/neu kinase-dependent modulation of androgen receptor function through effects on DNA binding and stability. *Cancer Cell* **6:** 517–27

Reagan-Shaw S, Ahmad N (2005) Silencing of polo-like kinase (Plk) 1 via siRNA causes induction of apoptosis and impairment of mitosis machinery in human prostate cancer cells: implications for the treatment of prostate cancer. *FASEB J* **19:** 611–13

Sanchez P, Hernandez AM, Stecca B et al (2004) Inhibition of prostate cancer proliferation by interference with SONIC HEDGEHOG-GLI1 signaling. *Proc Natl Acad Sci USA* **101:** 12561–6

Sanchez P, Clement V, Altaba A (2005) Therapeutic targeting of the Hedgehog-GLI pathway in prostate cancer. *Cancer Res* **65:** 2990–2

Sansal I, Sellers WR (2004) The biology and clinical relevance of the PTEN tumor suppressor pathway. *J Clin Oncol* **22:** 2954–63

Seligson DB, Horvath S, Shi T et al (2005) Global histone modification patterns predict risk of prostate cancer recurrence. *Nature* **435:** 1262–6

Varambally S, Dhanasekaran SM, Zhou M et al (2002) The polycomb group protein EZH2 is involved in progression of prostate cancer. *Nature* **419:** 624–9

Verras M, Sun Z (2005) Roles and regulation of Wnt signaling and beta-catenin in prostate cancer. *Cancer Lett* Epub (in press)

Vogelstein B, Kinzler KW (2004) Cancer genes and the pathways they control. *Nat Med* **10:** 789–99

Zhang M, Latham DE, Delaney MA, Chakravarti A (2005) Survivin mediates resistance to antiandrogen therapy in prostate cancer. *Oncogene* **24:** 2474–82