Lessons from in-vivo models of castration-resistant prostate cancer

Dong Lin[a,b], *Peter W. Gout*[b], *and Yuzhuo Wang*[a,b,c]

Purpose of review
Although the treatment of castration-resistant prostate cancer (CRPC) has benefited from the use of increasingly potent androgen synthesis inhibitors and androgen receptor (AR) antagonists, it is only marginally effective. There is therefore a critical need for a better understanding of the mechanisms underlying the CRPC development and more effective therapeutic approaches. Here, we focus on the advancements reported in the last 18 months, particularly with regard to the mechanisms of castration resistance and potential therapeutic targets emerging from the studies with in-vivo models.

Recent findings
Recent findings indicate that AR-dependent mechanisms, for example, increased expression of CYP17A1 and AR splice variants, play important roles in in-vivo castration resistance to new antiandrogens and androgen synthesis inhibitors. Whereas current therapeutic approaches focus on AR-dependent CRPC, studies based on genetically engineered mouse models indicate that castration resistance can develop in the absence of robust AR signaling. Furthermore, increasing evidence suggests that cellular plasticity of prostate adenocarcinoma allows AR-independent CRPC development via various adaptive mechanisms.

Summary
Significant progress has been made in the understanding of AR-dependent and AR-independent mechanisms involved in the development of CRPC. This may lead to identification of new therapeutic targets and improved therapy.

Keywords
androgen receptor, animal model, castration-resistant prostate cancer, cellular plasticity, neuroendocrine transdifferentiation

INTRODUCTION

Prostate cancer is the most commonly diagnosed noncutaneous cancer and the second leading cause of cancer-related death in North American men [1]. While androgen deprivation therapy (ADT) can induce marked tumor regression, resistance to treatment inevitably emerges, leading to castration-resistant prostate cancer (CRPC). There is evidence that CRPC can be driven by very small amounts of androgen, and new therapeutic approaches that more potently target androgen–androgen receptor (AR) signaling have been developed [2▪▪,3▪▪]. However, the mechanisms underlying the development of castration resistance are still not clear and treatment options for CRPC are limited. Although current therapeutic development focuses on the AR-dependent CRPC, it is hypothesized that the use of more potent ADTs will increase the occurrence of treatment-induced, AR-independent CRPC, such as neuroendocrine prostate cancer (NEPC), an aggressive, AR-independent prostate cancer subtype observed in 30–100% of CRPCs following androgen deprivation [4,5]. There is therefore a critical need for new, more effective approaches for the treatment of advanced prostate cancer. In this review, we focus on the advancements reported in the last 18 months, particularly with regard to the mechanisms of castration resistance and the

[a]The Vancouver Prostate Centre, Vancouver General Hospital, [b]Department of Experimental Therapeutics, BC Cancer Agency and [c]Department of Urologic Sciences, Faculty of Medicine, University of British Columbia, Vancouver, British Columbia, Canada

Correspondence to Yuzhuo Wang, PhD, Department of Experimental Therapeutics, BC Cancer Agency – Cancer Research Centre, 675 West 10th Avenue, Vancouver, BC, Canada V5Z 1L3. Tel: +1 604 675 8013; fax: +1 604 675 8019; e-mail: ywang@bccrc.ca

Curr Opin Urol 2013, 23:214–219
DOI:10.1097/MOU.0b013e32835e9f07

KEY POINTS

- There is evidence that continued activity of androgen–AR signaling remains an important mechanism underlying castration resistance to highly potent antiandrogen agents (e.g. enzalutamide) and androgen synthesis inhibitors (e.g. abiraterone).
- Whereas current therapeutic approaches focus on AR-dependent CRPC, AR-independent castration-resistant mechanisms bypassing the androgen–AR axis will likely develop following the use of increasingly potent anti-AR and antiandrogen biosynthesis therapies.
- PI3K/AKT/mTOR signaling can bypass the need for AR in PTEN null-mouse prostate cancer models, indicating castration resistance can develop in the absence of robust AR signaling.
- Increasing evidence suggests that the cellular plasticity of prostate adenocarcinoma allows AR-independent CRPC development via various adaptive mechanisms.

potential therapies that are emerging from studies with in-vivo models.

ANDROGEN-RECEPTOR-DEPENDENT MECHANISMS

Significant benefit from therapy with increasingly potent inhibitors of androgen synthesis and anti-androgen reagents has indicated the importance of AR-mediated signaling in castration resistance. Mechanisms underlying the treatment resistance can be divided into two categories: ligand-dependent AR signaling (e.g., alternative androgen production and AR gene amplification) and ligand-independent AR signaling (e.g., induction of AR splice variants, alterations of AR co-regulators and crosstalk of AR signaling with other signaling pathways).

Ligand-dependent androgen receptor signaling

Extratesticular androgen, produced by adrenal glands and apparently also *de novo* in CRPC, plays an essential role in therapy resistance of CRPC. Although a new, potent CYP17 inhibitor, abiraterone acetate (abiraterone), targeting 17α-hydroxylase/C17,20 lyase (CYP17A1) in testicular, adrenal and prostatic tumor tissues, has shown significant improvement in clinical outcomes, many patients do not show a PSA response, and progression still occurs despite treatment with this drug [3▪▪]. A better understanding of the mechanism(s) underlying abiraterone resistance is therefore important for the development of new therapies. In a recent study, increased expression of CYP17A1 was observed in abiraterone-treated LuCaP35CR and LuCaP23CR xenografts; as well, induction of steroidogenesis was observed [6▪]. Another study showed increased intratumoral expression of CYP17A1 in abiraterone-treated VCaP xenografts; upregulation of CYP17A1 was further confirmed in CRPC tissue biopsies from patients treated with a CYP17A1 inhibitor and a 5α-reductase inhibitor [7▪]. Together, these studies suggest that resistance to abiraterone may occur through the upregulation of CYP17A1 and induction of *de novo* androgen synthesis, and that treatment resistance of CRPC may be overcome by therapeutic approaches that can further suppress *de novo* intratumoral androgen synthesis.

In addition to abiraterone, other potent androgen biosynthesis inhibitors have been developed. TAK-700 (Orteronel) is a highly potent and selective 17,20-lyase inhibitor of CYP17A; it has shown high androgen synthesis-inhibitory capacity *in vitro* and in male monkey models [8], and is currently in a phase III trial. More compounds targeting androgen synthesis have recently been developed and will hopefully go into clinical trials.

Recently, a new mechanism of androgen biosynthesis enhancement has been discovered. Hyperinsulinemia is a consistent systemic side-effect induced by ADT, and using androgen-dependent prostate cancer in-vitro models, it was found that treatment with insulin upregulated the levels of intracellular testosterone and secreted androgens via induction of several key enzymes involved in *de novo* steroidogenesis. Furthermore, increased expression of the insulin receptor and its substrates was associated with the development of castration resistance in studies with LNCaP xenografts [9▪]. The results suggest that insulin can increase *de novo* androgen synthesis in CRPC via direct action on prostate cancer cells and further studies will likely reveal more potential targets for inhibiting androgen synthesis.

Clinical benefit from the new-generation anti-androgen, enzalutamide, suggests that development of more potent AR antagonists could improve patients' survival. Recently, a structural analog of enzalutamide, ARN-509, has been developed. It was reported to inhibit both AR nuclear translocation and AR binding to androgen response elements and to have greater efficacy in mouse CRPC models than enzalutamide, although *in vitro* it had a similar efficacy. ARN-509 is also expected to result in a higher therapeutic index than enzalutamide and reduce the risk of seizure, a major side-effect induced by treatment with the latter [10▪]. It is anticipated that ARN-509 may provide a new approach for the treatment of CRPC as a single agent or in combination with other agents that target key CRPC

driving pathways. Ongoing clinical trials will show whether it can achieve greater clinical benefit than enzalutamide.

Ligand-independent androgen receptor signaling

Driving of CRPC via AR signaling without androgen binding is a major problem in the clinic, as conventional hormone therapy in such a case is not effective. A number of processes have been shown to play an important role in ligand-independent AR signaling, including the development of AR splice variants, altered expression of AR coactivators/corepressors, and crosstalk between AR and other signaling pathways.

Androgen receptor splice variants

Expression of several AR splice variants (AR-Vs), that lack the ligand-binding domain of AR and are constitutively active, is emerging as a mechanism contributing to the development of CRPC. Recently, treatment of CRPC xenografts with enzalutamide was found to lead to increased expression of AR-Vs, but not full-length AR, suggesting that an adaptive shift toward AR-V-mediated signaling may be a mechanism underlying acquired resistance to enzalutamide [11▪]. Another independent study highlights AR-Vs as key mediators of persistent AR signaling and resistance to current AR-directed therapies, indicating potential usefulness of AR-Vs as therapeutic targets in advanced disease [12▪]. Furthermore, increased expression of AR-Vs has been reported to contribute to abiraterone resistance in LuCaP23 and LuCaP35 xenografts [6▪]. However, whereas one study has indicated that dimerization of AR splice variants with full-length AR is required for their functionality [13], some recent studies have shown that some of the AR-Vs are independent of full-length AR for full transcriptional activity [11▪]. The function of such AR-Vs would therefore not be effectively blocked by new, potent AR antagonists such as enzalutamide. The results of these studies indicate a reliance of CRPC on AR-V-mediated transcriptional pathways. They suggest that the combination therapy of novel agents targeting AR-V at the amino-terminal domain, such as EPI-001 [14], and agents targeting androgen biosynthesis could provide more complete blockage of AR signaling and hence evoke a more prolonged therapeutic response.

Androgen receptor coactivators and corepressors

AR coactivators and corepressors have been implicated in the development of CRPC. L-DOPA decarboxylase (DDC) is an AR coactivator that increases in the expression with disease progression and its coexpression with AR in prostate adenocarcinoma cells can enhance AR activity [15]. Recently, it was demonstrated that carbidopa, an FDA-approved DDC inhibitor for the treatment of Parkinson's disease, can abrogate DDC coactivation of AR in prostate cancer cells, reduce tumor growth and delay CRPC development in an LNCaP xenograft model [16]. Furthermore, combination therapy of bicalutamide and carbidopa has been shown to significantly delay CRPC progression *in vivo* [17]. Another study by Li *et al.* [18] reported that prostate leucine zipper (PrLZ) could function as an AR activator to transactivate AR target genes and increase AR-mediated prostate cancer cell growth. Targeting the AR–PrLZ complex with PrLZ-siRNA resulted in growth suppression of various human prostate cancer cell cultures and in-vivo mouse prostate cancer models. These results suggest that the use of AR coactivator-targeting drugs in combination with antiandrogens can improve the efficacy of the antiandrogens. Future studies, combining carbidopa or a PrLZ inhibitor with recently developed antiandrogens in clinically relevant animal models, may provide support for such combination therapy in the clinic.

Survival signaling pathways

Interaction of AR signaling with oncogenic pathways also plays a role in ligand-independent activation of AR. Loss of PTEN function, and consequently deregulation of PI3K/AKT signaling, is one of the most common alterations found in prostate cancers. However, the interaction between the PTEN/PI3K/AKT pathway and AR signaling and its role in CRPC development are not clear. Results of two recent studies, using conditional PTEN-knockout mouse models of prostate cancer, have indicated that PTEN deletion can reduce AR expression via different mechanisms. Studies by Carver *et al.* [19▪▪] indicated that loss of PTEN and activation of HER kinase can decrease AR activity. As demonstrated by Mulholland *et al.* [20▪▪] with genetically engineered mouse (GEM) models harboring conditional PTEN and AR knockouts, AR function is not a prerequisite for the development of prostate cancer. It was shown that loss of PTEN inhibited the expression and activity of a number of proteins involved in modulating AR activity and consequently repressed AR activity. Furthermore, AR-null cancers arising in PTEN null mice showed increased rates of cancer cell proliferation. Together, the data suggest that PTEN loss and AKT activation can suppress AR signaling, and that prostate cancer

development can occur in the absence of robust AR signaling. These experimental studies based on GEM models suggest that an AR-independent mechanism may underlie the development of CRPC. Furthermore, combination therapies targeting AR signaling and AKT/mTOR activity may improve disease management and patient survival.

Proliferative signaling pathways

Deregulation of certain genes and molecular pathways has been associated with prostate cancer progression, including activation of PI3K–AKT–mTOR and RAF–MEK–ERK–MAPK pathways and increased expression of MYC. However, the mechanisms by which MYC is activated and its interaction with other signaling pathways remain unclear. Recently, Wang *et al.* [21▪] have shown that upregulation of c-Myc cooperated with the activation of PI3K–AKT–mTOR and MAPK signaling pathways in a GEM model, in which conditional loss-of-function of PTEN was combined with the activation of oncogenic Braf. Accordingly, therapeutic treatments targeting these pathways attenuated c-Myc levels and reduced tumor and metastatic burden in the same system. It was suggested that Myc pathway activation is an important consequence of activation of PI3K–AKT–mTOR and MAPK signaling in advanced prostate cancer. These findings provide novel insights into Myc as a target for therapeutic intervention. It should be noted that the oncogenic mutation of BRAF and pattern of CRPC tumor development (lacking a regression phase after castration) in this model are rarely observed in the clinic [22,23]. Further validation of these findings in clinical studies is therefore needed.

Cytoprotective chaperone proteins

Cytoprotective proteins such as clusterin (CLU) and other heat shock proteins (HSPs) have been shown to contribute to CRPC progression. Small-molecule HSP inhibitors show promise in the treatment of CRPC; however, such HSP inhibitors trigger a heat shock response which is associated with increased expression of HSPs, including CLU, that attenuates their efficacy. Recent studies by Lamoureux *et al.* [24] showed that inhibition of CLU *in vitro* abrogates the heat shock response induced by Hsp90 inhibitors. *In vivo*, Hsp90 inhibitors in combination with OGX-011, an antisense CLU-targeting drug, markedly enhanced antitumor efficacy in xenograft models of human CRPC, leading to an 80% inhibition of tumor growth with prolonged survival compared with Hsp90 inhibitor monotherapy. This study indicates that strategies targeting CLU in combination with Hsp90 inhibitors can improve patient outcome in CRPC. Furthermore, CLU has been reported to mediate TGF-β-induced epithelial–mesenchymal transition (EMT) and its suppression was found to inhibit metastasis in an in-vivo prostate cancer model. As such, inhibition of CLU may represent a promising therapeutic option for suppressing prostate cancer metastatic progression [25].

ANDROGEN RECEPTOR-INDEPENDENT MECHANISMS AND CELLULAR PLASTICITY

AR-independent mechanisms will likely develop following the use of increasingly potent anti-AR and antiandrogen biosynthesis therapies. Following androgen ablation therapy, prostatic adenocarcinoma cells can display cellular plasticity which is evidenced by transdifferentiation processes such as EMT, neuroendocrine transdifferentiation and dedifferentiation into stem cell-like cancer cells. Such plasticity is also reflected in epithelial to immune cell-like transition (EIT), a proposed transdifferentiation process by which prostate cancer can overcome the immune response.

EMT was initially identified as a process of epithelial cells acquiring an invasive mesenchymal phenotype in morphogenesis and was subsequently found to promote cancer metastasis and treatment resistance. Recently, it was shown with LuCaP35 prostate cancer xenografts that androgen deprivation caused EMT as well as increased stemness in the surviving cancer cells. Similar changes were observed in prostate cancer samples obtained from ADT-treated patients [26▪]. The results of the study raise the possibility that EMT is an underlying mechanism of castration resistance. Future experiments will have to establish whether it provides a useful target for the treatment of CRPC.

NEPC does not express AR and therefore does not respond to androgen ablation. The cellular origin of NEPC and the molecular mechanisms involved in its development are largely unknown. Recently, we obtained experimental evidence for neuroendocrine transformation, using a patient's prostate adenocarcinoma tissue-derived xenograft model, which retains high fidelity to the cancer of origin (www.livingtumorlab.com). Several months after host castration, the recurrent tumor was entirely AR negative and exhibited a typical neuroendocrine phenotype as well as androgen-independent growth. The similarity of the gene copy number profiles of the original and recurrent xenografts indicates that a complete transformation of adenocarcinoma to NEPC had taken place via an adaptive mechanism (unpublished observation). The data suggest epithelial plasticity of conventional adenocarcinoma and also underscore the

usefulness of patient-derived xenografts for in-depth studies of the dynamic progression of neuroendocrine transdifferentiation. Another study demonstrated that a combination of such patient-derived cancer models with advanced genomic profiling techniques can lead to the discovery of key therapeutic targets and the development of therapies potentially useful for personalized chemotherapy [27▪]. The mechanism of NEPC development was also studied with patient tissue-derived NEPC xenograft models developed by Tzelepi *et al.* [28]. Gene expression and genomic profiling of NEPC xenografts compared with typical prostate adenocarcinoma xenografts revealed upregulation of mitotic genes, coupled to loss of AR, RB and cyclin D1 expression. These findings, validated immunohistochemically using a panel of clinical CRPC samples, suggest that the observed gene-expression changes contribute to the development and AR-independent growth of NEPC.

The EMT, neuroendocrine transdifferentiation, as well as EIT, a newly proposed mechanism underlying the immune-suppressive activity of epithelial cancers [29], are transdifferentiation processes aimed at the survival of prostate cancer cells that reflect their plasticity. Such ability suggests that adaptive mechanisms could underlie the development of castration resistance. In view of this, such adaptive responses could serve as a potential target for therapy.

CONCLUSION

Recent studies based on in-vivo models have provided additional evidence that continued activity of AR signaling is an important mechanism underlying castration resistance, even when highly potent antiandrogens and androgen synthesis inhibitors are used, such as enzalutamide and abiraterone. The findings suggest that combination therapy of novel agents targeting AR and androgen biosynthesis could improve the therapeutic response. Alternative resistance mechanisms independent of AR signaling will likely evolve following treatment with increasingly potent anti-AR and antiandrogen therapies, as suggested by the recent findings that PI3K/AKT/mTOR signaling can circumvent the need for AR signaling in PTEN null-mouse prostate cancer models. Increasing evidence suggests that the cellular plasticity of prostatic adenocarcinoma allows CRPC development and progression via various adaptive AR-independent mechanisms. A better understanding of the AR-independent mechanisms may lead to the identification of novel therapeutic targets for better management of the disease.

Acknowledgements

The research was supported by grants from the Canadian Institutes of Health Research, Prostate Cancer Canada and the BC Cancer Foundation. The authors sincerely apologize to all colleagues whose studies have not been referenced in this article.

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ▪ of special interest
- ▪▪ of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 287).

1. Jemal A, Bray F, Center MM, *et al.* Global cancer statistics. CA Cancer J Clin 2011; 61:69–90.
2. ▪▪ Scher HI, Fizazi K, Saad F, *et al.* Increased survival with enzalutamide in prostate cancer after chemotherapy. N Engl J Med 2012; 367:1187–1197.
In a phase III, double-blind, placebo-controlled, clinical trial, enzalutamide significantly prolonged the overall survival of patients with metastatic CRPC who previously received chemotherapy.
3. ▪▪ Fizazi K, Scher HI, Molina A, *et al.* Abiraterone acetate for treatment of metastatic castration-resistant prostate cancer: final overall survival analysis of the COU-AA-301 randomised, double-blind, placebo-controlled phase 3 study. Lancet Oncol 2012; 13:983–992.
The final analysis of the COU-AA-301 double-blind, placebo-controlled, phase III study shows that abiraterone acetate significantly prolongs the overall survival of patients with metastatic CRPC after docetaxel treatment.
4. Nelson EC, Cambio AJ, Yang JC, *et al.* Clinical implications of neuroendocrine differentiation in prostate cancer. Prostate Cancer Prostatic Dis 2007; 10: 6–14.
5. Palmgren JS, Karavadia SS, Wakefield MR. Unusual and underappreciated: small cell carcinoma of the prostate. Semin Oncol 2007; 34:22–29.
6. ▪ Mostaghel EA, Marck BT, Plymate SR, *et al.* Resistance to CYP17A1 inhibition with abiraterone in castration-resistant prostate cancer: induction of steroidogenesis and androgen receptor splice variants. Clin Cancer Res 2011; 17:5913–5925.
This study demonstrates that mechanisms of abiraterone resistance may include upregulation of CYP17A1 and upregulation of AR-Vs in LuCaP xenograft models.
7. ▪ Cai C, Chen S, Ng P, *et al.* Intratumoral de novo steroid synthesis activates androgen receptor in castration-resistant prostate cancer and is upregulated by treatment with CYP17A1 inhibitors. Cancer Res 2011; 71:6503–6513.
This study demonstrates the upregulation of CYP17A in abiraterone-treated VCaP xenografts and clinical samples following CYP17A1 inhibitor therapy, suggesting CYP17A upregulation is a mechanism underlying the resistance to treatment with abiraterone.
8. Kaku T, Hitaka T, Ojida A, *et al.* Discovery of orteronel (TAK-700), a naphthylmethylimidazole derivative, as a highly selective 17,20-lyase inhibitor with potential utility in the treatment of prostate cancer. Bioorg Med Chem 2011; 19:6383–6399.
9. ▪ Lubik AA, Gunter JH, Hendy SC, *et al.* Insulin increases de novo steroidogenesis in prostate cancer cells. Cancer Res 2011; 71:5754–5764.
This study provides evidence that high insulin levels can increase *de novo* androgen synthesis and hence contribute to castration resistance.
10. ▪ Clegg NJ, Wongvipat J, Joseph JD, *et al.* ARN-509: a novel antiandrogen for prostate cancer treatment. Cancer Res 2012; 72:1494–1503.
This study demonstrates that ARN-509 has greater efficacy than enzalutamide in mouse CRPC models, providing preclinical proof-of-principle for ARN-509 as a potential drug candidate for CRPC.
11. ▪ Hu R, Lu C, Mostaghel EA, *et al.* Distinct transcriptional programs mediated by the ligand-dependent full-length androgen receptor and its splice variants in castration-resistant prostate cancer. Cancer Res 2012; 72:3457–3462.
This study demonstrates that AR-Vs can activate gene-expression signatures distinct from full-length AR and indicates that AR-Vs-mediated signaling may be involved in the development of resistance to enzalutamide.
12. ▪ Li Y, Chan SC, Brand LJ, *et al.* Androgen receptor splice variants mediate enzalutamide resistance in castration-resistant prostate cancer cell lines. Cancer Res 2013; 73 [Epub ahead of print]. doi: 10.1158/0008-5472. CAN-12-3630.
This study demonstrates that AR-Vs resulting from AR gene rearrangement can mediate enzalutamide resistance in CRPC.

13. Watson PA, Chen YF, Balbas MD, *et al.* Constitutively active androgen receptor splice variants expressed in castration-resistant prostate cancer require full-length androgen receptor. Proc Natl Acad Sci USA 2010; 107:16759–16765.

14. Andersen RJ, Mawji NR, Wang J, *et al.* Regression of castrate-recurrent prostate cancer by a small-molecule inhibitor of the amino-terminus domain of the androgen receptor. Cancer Cell 2010; 17:535–546.

15. Wafa LA, Cheng H, Rao MA, *et al.* Isolation and identification of L-DOPA decarboxylase as a protein that binds to and enhances transcriptional activity of the androgen receptor using the repressed transactivator yeast two-hybrid system. Biochem J 2003; 375 (Pt 2):373–383.

16. Wafa LA, Cheng HL, Plaa N, *et al.* Carbidopa abrogates L-DOPA decarboxylase coactivation of the androgen receptor and delays prostate tumor progression. Int J Cancer 2012; 130:2835–2844.

17. Thomas C, Wafa LA, Lamoureux F, *et al.* Carbidopa enhances antitumoral activity of bicalutamide on the androgen receptor-axis in castration-resistant prostate tumors. Prostate 2012; 72:875–885.

18. Li L, Xie H, Liang L, *et al.* Increased PrLZ-mediated androgen receptor transactivation promotes prostate cancer growth at castration-resistant stage. Carcinogenesis 2012 [Epub ahead of print]. doi: 10.1093/carcin/bgs337.

19. ■■ Carver BS, Chapinski C, Wongvipat J, *et al.* Reciprocal feedback regulation of PI3K and androgen receptor signaling in PTEN-deficient prostate cancer. Cancer Cell 2011; 19:575–586.

This study demonstrates that loss of PTEN and activation of HER kinase can decrease AR activity in a mouse prostate cancer model and in human prostate cancer xenografts.

20. ■■ Mulholland DJ, Tran LM, Li Y, *et al.* Cell autonomous role of PTEN in regulating castration-resistant prostate cancer growth. Cancer Cell 2011; 19:792–804.

This study demonstrates that PTEN loss can suppress AR signaling and that prostate cancer development can occur in the absence of robust AR signaling.

21. ■ Wang J, Kobayashi T, Floc'h N, *et al.* B-Raf activation cooperates with PTEN loss to drive c-Myc expression in advanced prostate cancer. Cancer Res 2012; 72:4765–4776.

This study demonstrates that Myc pathway activation is an important consequence of activation of PI3K–AKT–mTOR and MAPK signaling in a GEM model.

22. Davies H, Bignell GR, Cox C, *et al.* Mutations of the BRAF gene in human cancer. Nature 2002; 417:949–954.

23. Cho NY, Choi M, Kim BH, *et al.* BRAF and KRAS mutations in prostatic adenocarcinoma. Int J Cancer 2006; 119:1858–1862.

24. Lamoureux F, Thomas C, Yin MJ, *et al.* Clusterin inhibition using OGX-011 synergistically enhances Hsp90 inhibitor activity by suppressing the heat shock response in castrate-resistant prostate cancer. Cancer Res 2011; 71:5838–5849.

25. Shiota M, Zardan A, Takeuchi A, *et al.* Clusterin mediates TGF-beta-induced epithelial–mesenchymal transition and metastasis via Twist1 in prostate cancer cells. Cancer Res 2012; 72:5261–5272.

26. ■ Sun Y, Wang BE, Leong KG, *et al.* Androgen deprivation causes epithelial–mesenchymal transition in the prostate: implications for androgen-deprivation therapy. Cancer Res 2012; 72:527–536.

This study demonstrates that androgen deprivation can cause EMT, as well as increased stemness in the LuCaP35 prostate cancer xenografts.

27. ■ Collins CC, Volik SV, Lapuk AV, *et al.* Next generation sequencing of prostate cancer from a patient identifies a deficiency of methylthioadenosine phosphorylase, an exploitable tumor target. Mol Cancer Ther 2012; 11:775–783.

This study demonstrates that use of a highly clinically relevant, patient-derived prostate cancer models in combination with advanced genomic profiling techniques can lead to the identification of therapeutic targets and therapies potentially useful for personalized chemotherapy.

28. Tzelepi V, Zhang J, Lu JF, *et al.* Modeling a lethal prostate cancer variant with small-cell carcinoma features. Clin Cancer Res 2012; 18:666–677.

29. Choi SY, Gout PW, Collins CC, Wang Y. Epithelial immune cell-like transition (EIT): a proposed transdifferentiation process underlying immune-suppressive activity of epithelial cancers. Differentiation 2012; 83:293–298.