

# Novel twists in hormone-mediated carcinogenesis

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This special issue of *Endocrine-Related Cancer* highlights key emerging concepts, novel twists and challenges in resistance to hormonal therapies for breast and prostate cancer. Emerging therapeutic strategies to overcome such resistance and advances in patient-derived xenografts to better model solid tumours are presented.

The growth of prostate cancer is exquisitely dependent on androgens and the androgen receptor (AR). Thus, the primary treatment option for men with metastatic prostate cancer is androgen deprivation therapy (ADT), which suppresses androgen biosynthesis and/or binding of ligand to the AR. Although most men initially respond to ADT, their cancer inevitably returns in an incurable and lethal form termed castration-resistant prostate cancer (CRPC) (Scher *et al.* 2004). The advent of new-generation AR-targeting agents, such as the AR antagonist enzalutamide and the androgen biosynthesis inhibitor abiraterone, has improved survival outcomes for men with CRPC (Suzman & Antonarakis 2014). However, the overall survival benefits associated with these new treatment strategies are in the order of months (Recine & Sternberg 2015), highlighting the capacity for AR signalling to adapt to all forms of ADT and the intractable problems associated with therapies that only inhibit ligand activation of the AR ligand binding.

Coutinho and coworkers specifically address key mechanisms underlying persistent AR signalling in CRPC and how this relates to resistance to AR target therapies (Coutinho *et al.* 2016). Although many such reviews are available in the literature, several novel features set this one apart from others. First, it comprehensively catalogues the spectrum of AR mutations and alternative splicing events identified in prostate cancer, including details on the frequency, function and tissue source of such alterations. Tabular summaries with this level of information are extremely useful and provide

an important resource for researchers working in this field. Second, the authors integrate a review of oncogenic functions of AR with a pertinent outline of its role in normal physiology, which likely is critical to understanding persistent AR signalling in an androgen-deplete environment. This provides a unique platform to explore the question ‘why is prostate cancer so reliant on AR signalling?’, a critical point that is rarely addressed in reviews on this topic. Coutinho and coworkers (Coutinho *et al.* 2016) also discuss the evolution of the AR signalling axis during disease progression in the context of two key concepts in oncology: oncogene addiction and therapy-mediated selection pressure. Finally, this review examines novel therapeutic strategies to inhibit resistance to androgen deprivation therapies, with consideration of the importance of targeting strategies distinct from the current focus on inhibiting ligand binding. For example, the authors discuss targeting the amino-terminal domain of the AR, which represents a particularly relevant target in CRPC because it is preserved in all forms of the AR that are resistant to current AR-targeting agents, including full-length AR (FL-AR) containing gain-of-function mutations as well as constitutively active AR-variants (AR-Vs).

Originally reported by Tindall and coworkers (Dehm *et al.* 2008), the potential clinical significance of ligand-independent AR-Vs in CRPC came to prominence with a publication by Luo and coworkers (Antonarakis *et al.* 2014) showing that the presence of AR-V7 splice variants in circulating tumour cells (CTCs) was associated with an absence of response to abiraterone or enzalutamide and poor survival overall. Although the findings of that study suggested that AR-V7 is an important biomarker of response to AR target therapies, the role of AR-Vs in resistance to androgen deprivation therapies, while an attractive proposition to explain this common clinical

scenario, remains to be conclusively established. An in-depth discussion of AR-Vs in CRPC is provided by Cao and coworkers (Cao *et al.* 2016), especially their mechanism of action with particular emphasis on differences in the requirements of AR-Vs compared to FL-AR for specific co-regulators involved in determining their activity. The potential utility of AR-Vs as biomarkers of prognosis and treatment response, as well as their role in the emergence of resistance to endocrine therapies, is discussed. Although most AR-Vs act as positive regulators of transcription, this review covers less well-known actions of AR-Vs, including their localisation and function within the cytoplasm and at the plasma membrane, where AR can also act to modulate signalling. Cao and coworkers (Cao *et al.* 2016) describe the mechanisms whereby AR-Vs are generated, the regulation of their transcription by dimerisation partners and cofactors, as well as the role of splicing factors and chaperones. How cofactors could alter the abundance and activity of AR-Vs vs FL-AR is discussed, especially in the context of how the same cofactor could bind FL-AR and AR-Vs on different interfaces with different affinities. This highlights the complexity that confronts the field in attempting to target AR-Vs as a novel prostate cancer therapy. The authors propose that antagonism, transcriptional repression and interference with co-regulators are viable therapeutic approaches to target AR-Vs. The review concludes with an overview of recent preclinical compounds that have been shown to modulate AR-V activity.

Cyclin-dependent kinase 9 (CDK9) controls transcription in normal and malignant cells by regulating the phosphorylation state of RNA polymerase II. As discussed above, resistance to new generation therapies targeting AR in prostate cancer is common due to adaptive mechanisms that promote tumour cell survival in an androgen-depleted environment. Many such adaptive mechanisms occur at the transcriptional level. In this special issue, Rahaman and coworkers (Rahaman *et al.* 2016) provide an overview of the evidence that inhibition of CDK9 has a dual role in suppressing prostate cancer growth: it can reduce the constitutive expression of anti-apoptotic proteins (Li *et al.* 2000) and inhibit CDK9-mediated phosphorylation of the AR, which can promote its transcriptional activity (Chen *et al.* 2012). Rahaman and coworkers propose that CDK9 inhibition may overcome disease progression in CRPC by co-targeting the AR and anti-apoptotic proteins (Rahaman *et al.* 2016). The challenge to implement this strategy has been the lack of specific CDK9 inhibitors (CDK9is). Recent developments in the field suggest that selective

inhibition of CDK9 is achievable (Scholz *et al.* 2014), which undoubtedly will renew interest in the use of CDK9 inhibitors in combination with current standard-of-care therapies to improve the outcome of patients with CRPC. The success of this co-targeting approach in prostate cancer likely will depend on whether CDK9 inhibition is effective in inhibiting the activity of both FL-AR and aberrant forms of the AR (AR-Vs and mutant AR) that emerge in therapy-resistant tumours.

Approximately 75% of breast cancers are oestrogen receptor- $\alpha$  (ER) positive. Drugs that inhibit ER activity are the mainstay of adjuvant treatment for breast cancer and have improved disease outcomes, but resistance to current standard-of-care ER target therapies such as aromatase inhibitors (AIs) and tamoxifen is common (Burstein *et al.* 2014). Developing new drugs that extinguish ER activity is the current vogue to improve treatment outcomes. Recent work (Mohammed *et al.* 2015) supports a novel approach, whereby the activation of the progesterone receptor (PR) redirects oestrogen-stimulated ER to genomic loci associated with better disease outcomes. In that study, it was also revealed that PR loss is a common event, especially in the luminal B subtype of ER-positive disease. PR loss would potentially preclude some women from a PR-target therapy. However, this may be compensated for by the activation of the AR, which can also reprogram ER DNA binding and alter transcription to halt ER-positive tumour growth (Hickey *et al.* 2012).

In this special issue, Lim and coworkers (Lim *et al.* 2016) provide a timely review about targeting the interplay between sex steroid receptors as a therapeutic strategy in breast cancer. The authors specifically address the therapeutic potential for activating PR or AR in ER-positive breast cancer to redirect ER chromatin binding, i.e. 'pushing ER around' to elicit a transcriptional response associated with a good prognosis. Although PR- and AR-directed therapies have historically been used with reasonable success as endocrine therapies in patients with advanced breast cancer, these hormonal approaches have largely been abandoned in the clinic in favour of new-generation agents that more effectively eliminate ER activity. The authors review the preclinical breakthroughs utilising novel technologies to interrogate the interplay between these sex steroid hormones and summarise the increasing clinical interest in therapies that modulate these receptors.

Although new targeted therapies (e.g. PI3K and CDK pathway inhibition strategies) have made headway in the treatment of ER-positive breast cancer, the increasing costs of such strategies in combination with

current standard-of-care treatments mean that these therapies become cost-prohibitive, and alternative and more affordable options are still needed. Progestogens used to activate PR and, more recently, selective AR agonists, have been shown to be safe and well tolerated, raising the potential for an affordable alternate therapeutic approach that selectively harnesses the ability of sex steroid receptors to push ER towards anti-tumorigenic activity.

The role of PR and AR in ER-positive breast cancer is context dependent, with PR- and AR-directed therapies potentially taking the form of agonists and antagonists (Hickey *et al.* 2012, Singhal *et al.* 2016), making the presence of the receptor alone insufficient to determine the optimal therapeutic strategy. The review by Lim and coworkers (Lim *et al.* 2016) highlights the need to tease out the interplay between these sex steroid receptors and ER to define the preclinical rationale and identify the appropriate clinical context to evaluate the therapies targeting PR and AR.

Apart from ER, the other major biomarker and therapeutic target in breast cancer is the ERBB2 protein (also known as HER2/neu) encoded by the *ERBB2* gene. The review by Elizalde and coworkers (Elizalde *et al.* 2016) in this issue re-ignites a poorly appreciated discovery that is ripe for exploitation, namely the presence of nuclear ERBB2 in breast cancer and the potential role of nuclear ERBB2 in resistance to ERBB2-directed therapies. The authors provide a brief historical outline of both membrane-bound and nuclear ERBB2, an outline of ERBB2 structure and homology to ERBB1, the canonical downstream signalling pathways and response to ERBB2-directed therapies. The nature of ERBB2 target therapy resistance mechanisms and the need to develop strategies to circumvent resistance are particularly important as currently there are limited treatment options for this subset of breast cancer patients. The process of nuclear translocation of ERBB2 and its regulation by PR ligands is described. Elizalde and coworkers (Elizalde *et al.* 2016) further discuss ERBB2 interactions with other nuclear proteins such as STAT3 on chromatin.

The role of nuclear ERBB2 in resistance to currently employed chemotherapies and ERBB2-directed therapies is also addressed in this article, highlighting the potential of nuclear ERBB2 as a novel therapeutic target in breast cancer. Finally, the authors touch on the dearth of data that are available on the clinical significance of nuclear ERBB2.

The final review in this special issue addresses the importance of developing better models of

hormone-dependent cancers. While recognising the important scientific contributions made using cell lines and primary tissue 2D and 3D culture systems, as well as genetically modified mouse models, Cassidy and coworkers (Cassidy *et al.* 2016) emphasise the specific limitations and drawbacks of many models currently used in preclinical pipelines and drug discovery processes. The authors highlight the influence of factors such as the extracellular matrix on the biology of cancer cells and introduce the recent evolution of patient-derived tumour xenograft (PDX) models. The review highlights how PDX models better encapsulate the nature of human breast cancers such as tumour heterogeneity. It is envisaged that these models will more accurately reflect drug responsiveness, thereby providing a better platform for screening drugs and developing biomarkers of treatment response. Cassidy and coworkers (Cassidy *et al.* 2016) provide a thought-provoking discussion of their experience with establishing a PDX bank. They also propose that using short-term cultures of cells disaggregated from the PDX tumours (i.e. PDX tumour cultures or PDXc) could afford clinical practicality to PDX as a broadly utilised, robust and high-throughput preclinical model system for breast cancer drug discovery and screening. For example, this model system would greatly facilitate the evaluation and enhance implementation of emerging therapies for targeting PR and AR in ER-positive breast cancer as discussed in this special issue.

There is an urgent need to markedly improve survival of women and men with metastatic breast or prostate cancers that remain dependent on cognate hormone signalling pathways but are refractory to conventional sex steroid receptor target therapies. The collection of papers in this special issue of *Endocrine-Related Cancer* highlights the issues and challenges for the field to achieve this goal while at the same time providing insight into new approaches that may yield more durable treatment responses.

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#### Declaration of interest

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