

## PRECISION MEDICINE

# Sequence of events in prostate cancer

**Whole-genome sequencing reveals the duplication of a regulatory region, called an enhancer, of the *AR* gene in treatment-resistant human prostate cancers. The finding shows the importance of analysing non-protein-coding regions of DNA.**

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The publication<sup>1,2</sup> of the human genome sequence in 2001 was accompanied by optimism that a rise in the availability of genomic data might improve clinical treatments. It was hoped that such data might one day enable an approach termed ‘precision medicine’, in which therapies are tailored to target the abnormalities specific to a particular cancer. Since then, technological advances in DNA-sequencing techniques, combined with substantially lower costs, have led to a boom in the sequencing of cancer samples. Given this progress, one might assume that the key genetic alterations that drive common cancers are already well known. However, writing in *Cell*, Takeda *et al.*<sup>3</sup>, Viswanathan *et al.*<sup>4</sup> and Quigley *et al.*<sup>5</sup> detail a previously unidentified type of genetic alteration that frequently occurs in late-stage human prostate cancer.

The Cancer Genome Atlas (TCGA) and the International Cancer Genome Consortium (ICGC) have undertaken some of the largest-scale projects reported so far to sequence the DNA of human cancers. These efforts have identified many DNA alterations that drive cancer growth, including mutations and genomic rearrangements. TCGA has sequenced the protein-coding regions of approximately 11,000 individual genomes and 33 types of cancer (<https://portal.gdc.cancer.gov>), whereas the ICGC has sequenced the protein-coding regions from more than 20,000 individual genomes and 22 kinds of cancer (<https://dcc.icgc.org>). Both projects have focused mainly on sequencing the protein-coding regions of genes, which represent less than 2% of the entire genome. In the Pan Cancer Analysis of Whole Genomes (PCAWG) project, the ICGC and TCGA systematically analysed whole-genome sequencing data from many types of cancer. These data allowed scientists to investigate alterations in DNA regions that regulate gene expression, and in untranslated parts of gene sequences. This revealed that, in cancer cells, alterations in these non-protein-coding regions of DNA

occur at a similar frequency to those in the protein-coding regions<sup>6</sup>.

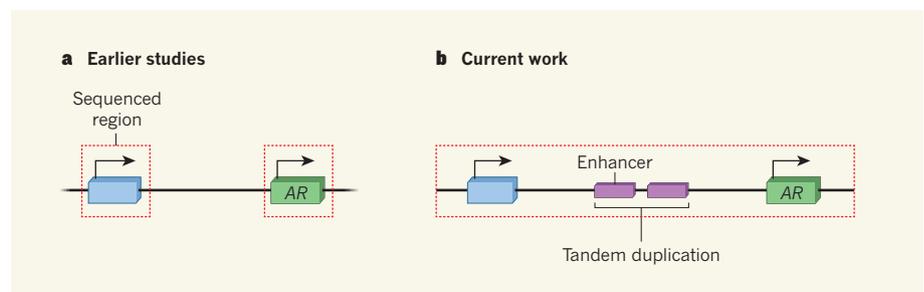
Many of the sequencing studies reported by TCGA and the ICGC focused predominantly on tumour samples taken from patients before cancer treatment. The work of Takeda, Viswanathan, Quigley and their respective colleagues provides some information about genetic alterations present in prostate cancers that are resistant to clinical treatment.

Prostate-cancer growth is usually driven by signalling pathways that act through the androgen receptor (AR), and a standard clinical treatment for advanced prostate cancer is to reduce the level of androgen hormones that activate ARs. Although this limits cancer growth for a while, tumours eventually become resistant to this therapy, and a highly malignant form of the cancer arises that is usually lethal. Such a tumour can migrate to other sites in the body through a process known as metastasis, and this sort of late-stage, treatment-resistant tumour is called a metastatic, castration-resistant prostate cancer.

When treatment resistance occurs, an

altered version of the gene that encodes AR is commonly found in the tumour. Mutations of the *AR* gene or amplifications of DNA that increase the copies of sequence encoding *AR* might enable tumour cells to enhance AR-pathway signalling even when androgen levels are low<sup>7,8</sup>. Analyses of protein-coding-sequence changes linked to prostate cancer have found alterations in *AR*, as well as in other known cancer-promoting genes<sup>9</sup>. Although a wealth of DNA sequencing data of protein-coding regions are available for prostate-cancer samples, there are comparatively few whole-genome sequences (only approximately 200 have been reported by the PCAWG project, for example; <https://dcc.icgc.org/pcawg>), and still fewer whole-genome sequencing data are available for metastatic, castration-resistant prostate cancer.

Takeda *et al.* re-evaluated previously published data<sup>10</sup> from clinical samples of castration-resistant prostate cancer and identified repeated DNA sequences that caused abnormal amplification of the region upstream of *AR* (Fig. 1). The authors describe this region as a type of gene-regulatory element called an enhancer, which is a sequence that can help to promote gene expression. When Takeda and colleagues used a genome-editing technique to target and suppress this region in human prostate-cancer cells grown *in vitro*, both cell proliferation and *AR* expression were reduced. The authors also engineered prostate-cancer cells grown *in vitro* to contain a duplication of the enhancer, and found that such cells showed increased *AR* expression and decreased sensitivity to an AR-targeting drug called enzalutamide that is used to treat metastatic, castration-resistant prostate cancer.



**Figure 1 | Duplication of an enhancer region of the genome occurs frequently in prostate cancer.**

**a**, Many of the earlier DNA-sequencing studies of human prostate cancer have focused on the protein-coding regions of the genome, such as the gene shown in blue (the red dotted box indicates sequenced regions). This work identified alterations in the sequence of the *AR* gene, which encodes the androgen receptor (AR), as being a common driver of disease progression<sup>7,8</sup>. **b**, Takeda *et al.*<sup>3</sup>, Viswanathan *et al.*<sup>4</sup> and Quigley *et al.*<sup>5</sup> demonstrate the utility of sequencing approaches that are not restricted to the protein-coding regions, and the advantages of sequencing tumour samples that have become resistant to therapy as a way of investigating why clinical treatment eventually fails. The three studies of late-stage prostate cancer report that the DNA sequence in a region upstream of *AR*, termed an enhancer, is commonly expanded, and this amplification is often in the form of a tandem duplication of the sequence. An enhancer amplification can drive expression of *AR*, which would enable tumours to evade the effects of clinical treatments that target the AR signalling pathway.

Viswanathan *et al.* present whole-genome sequencing data for 23 samples of metastatic, castration-resistant prostate cancer from patients. The authors compared this sequencing data with matched data from non-cancer cells from these individuals. This enabled the researchers to report alterations that characterize this type of prostate cancer. These included numerous duplicated sequences, with many duplications occurring in a tandem pattern. These tandem duplications frequently occurred in genome sequences adjacent to *AR* and to another cancer-promoting gene called *MYC*. The main region of sequence amplification associated with *AR* was in the same enhancer region that was identified by Takeda and colleagues. Viswanathan and colleagues found that the enhancer amplification was present in 87% of their samples, either with or without an amplified copy of *AR*.

Quigley and colleagues performed whole-genome sequencing of 101 samples of metastatic, castration-resistant prostate-cancer tissue obtained from previous studies<sup>11,12</sup>. The most frequently altered genomic site identified was the *AR*-enhancer region, which was amplified in 81% of samples. The high prevalence of this type of amplification is notable because enhancer amplifications identified so far for other cancer types generally arise at much lower frequency<sup>13–16</sup>. Moreover, the high prevalence of this *AR*-enhancer amplification in the data presented by Viswanathan and Quigley contrasts with its occurrence in only 1 of 54 previously published whole-genome sequences of prostate-cancer samples obtained before clinical treatment had commenced<sup>17</sup>. However, given the relatively small number of these whole-genome sequences from before treatment, it remains to be determined whether amplification of the *AR*-enhancer region usually arises at the time when cancers become treatment resistant, or

whether this alteration is already present in a subset of tumour cells before the cancer stops responding to treatment.

Quigley *et al.* did not report any correlation between the presence or absence of the *AR*-enhancer amplification and whether the cancer had progressed to the stage at which the patient received a second type of anti-androgen-pathway treatment, such as enzalutamide, after the first line of therapy had failed. Viswanathan *et al.* present sequencing data from three patients for whom tumour samples were available from before and after second-line anti-androgen-pathway treatment with enzalutamide; these data reveal that the samples from after treatment had an amplification of *AR* and the *AR* enhancer. If additional data from patients indicate a connection between the amplification of the *AR* enhancer and the emergence of treatment resistance, perhaps this amplification could be monitored as a biomarker of disease progression. Viswanathan and colleagues demonstrated that such alterations could be tracked through analysis of tumour DNA that is shed into patients' bloodstreams.

These studies highlight three important aspects of the way in which genomic analysis could illuminate our understanding of how cancers develop resistance to therapy. First, studying tumour samples obtained from patients before and during treatment might be the best way to understand how treatment resistance develops. Second, analysing a series of patient samples — such as biopsies or tumour DNA isolated from blood samples — during the course of therapy might help to reveal whether crucial DNA alterations arise during treatment or were already present in a subset of tumour cells before treatment. Quigley and colleagues' work with a large number of patient samples only partially addresses this. Analysis of patients over time

might also help to determine when therapy needs to be altered to try to prevent the development of treatment-resistant disease. Third, the technologies available for detecting genomic changes are rapidly improving, and the sequencing approaches used in the current studies can detect complex DNA alterations that were particularly challenging to determine using earlier techniques.

The genomic revolution that started with the Human Genome Project is reaching the cusp of a wave of detailed genomic studies that investigate how cancer evolves during treatment. Such progress represents another step closer to an era of precision medicine for cancer therapy. ■

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