DOT1L-HES6 fusion drives androgen independent growth in prostate cancer

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Molecular therapies targeting the androgen receptor (AR) or pathways involved in androgen synthesis form a critical component of the standard-of-care in treating aggressive, non-localized prostate cancers. The major problem with these therapies is that castration resistant clones arise within 1-3 years of treatment initiation, leading to clinical relapse and eventual death. Previously reported mechanisms of castration resistance include amplification and mutation of AR (Taplin et al., 1995; Visakorpi et al., 1995), neuroendocrine differentiation (Beltran et al., 2011), and aberrant activation of the glucocorticoid receptor (Arora et al., 2013). In a previous issue of this journal, Ramos-Montoya et al., 2014 implicated the transcription factor HES6 as another important player in the induction of castration resistance. In this correspondence, we present further evidence for the role of HES6 in castration resistant prostate cancer. We report a case of AR-negative prostate cancer driven by a DOT1L-HES6 fusion gene which directly induces overexpression and pathological activation of HES6.

We set out to study late stage prostate cancer by performing whole transcriptome and genome sequencing of two AR-negative prostate cancers from distinct patients. Sample #1 was obtained at prostatectomy from a 53-year-old patient with a Gleason 5+5 non-metastatic tumor and a prostate specific antigen (PSA) serum level of 4.8 μg/l. The tumor cells expressed high levels of ASCL1, CHGA, SYP, and HES6, four classical markers of neuroendocrine prostate cancer (Fig 1A) (Beltran et al., 2011). Sample #2 was obtained by transurethral resection of the prostate (TURP) from a 70-year-old patient originally diagnosed with a Gleason 4+5 non-metastatic tumor with a PSA of 62 μg/l. The diagnostic biopsy was positive for AR and ERG expression and negative for CHGA (Fig 1B). The patient was treated with orchietomy immediately after diagnosis and did not undergo prostatectomy. The TURP sample was taken 13 months after orchietomy and was negative for AR and ERG expression. The patient had a positive bone scan and a PSA of 1.1 μg/l when the TURP was performed, and died of his cancer 1 month later (14 months after orchietomy). Interestingly, the TURP sample did not show elevated expression of CHGA, SYP, or ASCL1, but did show strong HES6 expression (Fig 1A). Both samples were negative for MYCN and AURKA amplification.

To study the TURP sample further, we used ChimeraScan (Iyer et al., 2011) and an in-house algorithm to search for evidence of gene fusions in the transcriptome and whole genome sequencing data. Both algorithms identified a novel DOT1L-HES6 fusion gene, caused by an interchromosomal rearrangement that fused intron 9 of DOT1L with a position 4 kb upstream of HES6, resulting in HES6 overexpression (Fig 1C). HES6 is a member of the basic helix-loop helix (bHLH) family of transcription factors, and its expression is driven by ASCL1 in differentiating neurons (Nelson et al., 2009; Webb et al., 2013). HES6 was highly expressed in neuroendocrine prostate cancer models NCI-H660, LuCaP-49, and LuCaP-93, with concomitant high ASCL1 expression (Fig 1A). Among all AR-negative tumors we tested, the DOT1L-HES6 positive TURP sample from patient #2 was unique in having high HES6 but no ASCL1 activity (Fig 1A). This led us to hypothesize that the DOT1L-HES6 fusion results in ASCL1-independent activation of HES6, which in turn promotes androgen independent growth. To test whether HES6 overexpression induced androgen independence, we transfected androgen responsive LNCaP cells with a HES6 vector, resulting in 28-fold overexpression of HES6 relative to cells transfected with empty vector (P = 0.0173, unpaired two-tailed t-test, n = 2) (Fig 1D). We then grew the cells in mediums with different DHT levels and observed that HES6-transfected cells were able to grow in DHT concentrations as low as 0 nM (P = 9.6e-27, two-way analysis of variance, n = 4) and 1 nM (P = 4.4e-11, two-way analysis of variance, n = 4), while LNCaP cells transfected with empty vector were unable to grow in DHT-depleted mediums (Fig 1D). This finding is in agreement with the HES6 overexpression phenotype reported by Ramos-Montoya et al.

The diagnostic biopsy of patient #2 was negative for DOT1L-HES6 and HES6 expression based on qRT-PCR, indicating that the fusion gene had originated post-orchietomy (Fig 1E). To show that the DOT1L-HES6 positive TURP sample did not represent a new and independent tumor, we used the sequencing data to search for vestigial evidence of the ERG fusion present in the original diagnostic biopsy. Whole genome sequencing revealed a characteristic three
megabase deletion between the genes TMPRSS2 and ERG in chromosome 21 in the TURP sample (Fig 1F). Transcriptome sequencing also identified residual TMPRSS2-ERG expression in the TURP sample, although expression was very weak due to minimal AR activity.

In their publication, Ramos-Montoya et al proposed a model in which HES6 promotes androgen independence by modulating AR binding. The lack of AR activity in our DOT1L-HES6 fusion positive sample may indicate the existence of additional, AR-independent mechanisms. An alternative hypothesis is that the DOT1L-HES6 fusion in the TURP sample of patient #2 promoted castration resistance at an intermediate stage of tumor evolution, but was later subsumed by another mechanism that additionally resulted in complete loss of AR expression. Nonetheless, the lack of ASCL1, CHGA, and SYP overexpression distinguishes this tumor from classical neuroendocrine prostate cancers and highlights the role that HES6 plays in castration resistant and androgen independent tumors. This finding also calls for a more extensive search for HES6 genomic alterations in cohorts of AR-negative and castration resistant prostate cancers.

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Author contributions
MA, KK, and MN conceived and designed the experiments. MA performed computational and statistical analysis of the data. KK, KL, and JT performed wetlab experiments. MA and KK wrote the manuscript. MA, KK, WZ, and MN discussed and reviewed the manuscript.

Conflict of interest
The authors declare that they have no conflict of interest.
References

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