RB1 Depletion in Metastatic Castration Resistant Prostate Cancer: Novel Genomic Mechanisms and Clinical Impact

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Background: Copy number array and exome sequencing studies have illuminated genomic landscape of PCa. However, these data are of limited use to fully detect structural variations. Among commonly altered genes in metastatic castration resistant prostate cancer (mCRPC), RB1 is under scrutiny for its role in androgen-deprivation resistance and in adenocarcinoma dedifferentiation and/or transdifferentiation into high-grade neuroendocrine carcinomas (HG-NECs). Fusion transcripts involving RB1 have been reported, but frequency, heterogeneity and impact on protein expression is unclear. Of clinical importance, functional RB1 is necessary for CDK4/6 inhibitors to be effective.

Methods: We used deep whole genome sequencing (WGS) in a cohort of 21 mCRPCs from 10 men. WGS analyses were performed with a dedicated computational pipeline to detect allele specific events and map intra-patient heterogeneity. We also investigated RB1 copy number alterations (CNA) by a dual-color fluorescent *in situ* hybridisation (FISH) and protein expression by immunohistochemistry (IHC). RB1 FISH and IHC were evaluated in an additional cohort of 85 mCRPC samples from 85 patients. To determine an association between RB1 expression and proliferation, Ki-67 IHC staining was used.

Results: We identified copy number neutral loss-of-heterozygosity (LOH) for RB1 in 5 (5/21; 23.8%) and mono-allelic loss in another 5 (5/21; 23.8%) mCRPCs. Biallelic deletions were not detected in WGS data. Structural variants (SVs) involving RB1 were found in 3 patients (3/10; 30%), corresponding to 6 of the 21 tumours studied with WGS (28.57%), including previously unreported loss of expression due to tandem duplication. In two patients, corresponding to 5 out of 6 (83.33%) tumours, SVs were accompanied by complete protein loss on IHC and were shared by different metastases. The remaining patient with preserved IHC expression presented with copy number gains of the RB1 locus and 2 distinct SVs in a single metastasis. Heterogeneity for genomic losses and single-nucleotide variants were identified in 2 patients (2/10; 20%). We further evaluated this heterogeneity with RB1 IHC in 85 and FISH in 52 samples. Overall, in the 106 mCRPCs from 95 patients, RB1 IHC loss was heterogeneous in 30 samples (28.3%). Complete loss was seen in 8.33% (8/96) of adenocarcinomas and in 87.5% (7/8) of HG-NECs. FISH analyses identified CNAs in 48% (25/52) of the samples with overall rare cells showing biallelic deletions. Ki-67 IHC of mCRPC samples was prognostic on univariable analysis but RB1 IHC was not prognostic and did not correlate with Ki-67.

Conclusions: Genomically, RB1 LOH, monoallelic loss, and structural variants are common in mCRPCs; biallelic losses are rare. Dedicated assays are needed to cover the spectrum of RB1 somatic events. At the protein level, heterogeneous expression on IHC was observed in over a quarter of the samples. Our findings suggest that loss of RB1 protein is probably, in most cases, a late event. Clinically, our data is relevant to ongoing clinical trials of CDK4/6 inhibitors and implies careful patient selection is required.

Conflicts of Interest: The authors have no conflicts of interest to declare.

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