ATM loss in metastatic prostate cancer

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BACKGROUND: mCRPC can harbour homologous recombination mediated DNA repair gene defects; *ATM* aberrations are second commonest after BRCA2, present in approximately 5-7%. ATM loss of protein expression may impact DNA damage responses differently to dysfunctional ATM protein. We correlated ATM genomic aberrations with protein expression, studying biomarkers of ATM downstream inactivation, in a cohort enriched for cases with ATM gene defects.

METHODS: DNA was extracted from FFPE tumour blocks, using the QIAamp DNA Tissue kit (Qiagen). Libraries for amplicon-based targeted NGS were constructed using a customized Generead DNAseq Mix-n-Match Panel v2 (Qiagen) and were read on the MiSeq (Illumina). For the ATM IHC assay a rabbit monoclonal anti-ATM antibody Y170 (Abcam, Cambridge, UK) was used. For the pCHK2 assay, IHC was performed using the rabbit pChk2 monoclonal antibody clone C13C1 (Cell Signalling). IHC was evaluated by a pathologist using a semiquantitative H-score (0-300). A nuclear H-score of ≤10 was defined as negative.

RESULTS:
We assessed ATM in 355 selected prostate cancer samples from 285 patients, including 122 primary pre-treatment prostate biopsies, 13 prostatic biopsies after castration-resistance and 220 metastatic mCRPC biopsies. In all of 55 cases with paired (same patient) primary and CRPC samples, ATM loss/presence by IHC was concordant.

ATM expression by IHC was studied in 38 cases with suspected pathogenic ATM mutations or biallelic deletion. 31/38 (81.5%) cases had negative ATM IHC. The 7 cases without ATM loss of expression included 5 cases with truncating mutations beyond the diagnostic antibody-binding site, and 2 cases with truncating mutation but no LOH.

Next, we evaluated ATM IHC in 225 other cases with no targeted NGS aberrations; 16/225 (7.5%) had negative ATM IHC, suggesting there are additional mechanisms of gene inactivation not captured by targeted NGS.

The pCHK2 Ab was tested in cell lines with known absent/present ATM expression, showing concordance between ATM and pCHK2 loss. The pCHK2 IHC assay was then tested in a pilot cohort of 43 fresh mCRPC biopsies, enriched for cases with ATM loss. Lack of ATM and pCHK2 expression was associated (Chi-squared p-value: 0.024). Absence of pCHK2 had 69% sensitivity and 67% specificity to translate ATM null expression. Samples with preserved ATM but negative pCHK2 expression included 1 case with pathogenic *CHEK2* mutation and 2 cases with *CHEK2* deletions.

CONCLUSIONS: ATM loss is common in mCRPC samples. In 1/5 cases with ATM pathogenic mutations, there is protein expression by IHC. Cases with loss of protein expression without mutations detected were identified, suggesting other mechanisms of ATM inactivation occur in mCRPC. Validation by alternative sequencing and transcriptome methods will be pursued. Further assessment of CHK2 phosphorylation as indirect marker of ATM inactivation is being conducted.

Conflicts of interest: none

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