Neuroendocrine, amphicrine and AR$^+$ disease across lesions in a single mCRPC patient reveals a link between plasticity and clonal evolution

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Background: Metastatic castration-resistant prostate cancer (mCRPC) is clinically highly heterogenous. Genomic studies have revealed limited intra-patient genetic diversity, and key genetic events, such as *AR* amplifications, *TMPRSS2-ERG* fusions, and *TP53*, *RB1* and *PTEN* loss, are not enough to predict diverse disease biology. Epigenetic regulation and plasticity is considered fundamental in tumor adaptation and the development of AR-independent disease, including neuroendocrine (NE), AR^{low} and AR$^-$ disease phenotypes. However, we still do not understand what governs these changes and how they persist over time and across lesions despite distinct tumor microenvironments. Here, we investigated the link between plasticity, clonal evolution and the microenvironment in a mCRPC patient with highly heterogeneous disease.

Methods: Metastatic lesions of a patient with mCRPC who had previously received ADT, enzalutamide and cabazitaxel were obtained after death as part of the rapid-autopsy program at the Peter MacCallum Cancer Centre in Australia. Pathology review revealed heterogeneous disease, with AR$^+$ poorly differentiated adenocarcinoma disease in the lymph nodes, and AR$^-$, synaptophysin$^+$ and chromogranin A$^+$ undifferentiated carcinoma in the liver. PSMA-PET and FDG-PET scans obtained three months prior to death, showed high FDG uptake and low PSMA uptake in the liver lesions and the opposite phenotype lymph nodes. We performed single-nucleus (sn) multiome (RNA seq and ATACseq) of 3 metastatic sites (hilar lymph node, right and left liver lesions) and matched whole-genome sequencing.

Results: We identified multiple distinct tumor populations and clones in each metastatic lesion. Phenotypes based on canonical markers were linked to specific clones. All liver tumor clones were AR$^-$NE$^+$ and were clonally related to a common ancestor. In contrast, the hilar lymph node lesion was composed of two tumor clones, one being AR$^+$NE$^-$ and the other AR^{low}NE$^+$. The AR^{low}NE$^+$ clone was not a descendant of the AR$^-$NE$^+$ or AR$^+$NE$^-$ clones, suggesting that NE$^+$ disease could have potentially emerged independently multiple times. AR^{low}NE$^+$ cells had a combination of gene regulatory networks active in both AR$^+$NE$^-$ and AR$^-$NE$^+$ cells, however, do not represent a transitional state between both as these were clonally distinct. There were no point mutations or copy-number changes unique to the NE$^+$ clones that could distinguish them from the AR+ clones, suggesting the acquisition of NE features was likely epigenetic.

Conclusions: Features associated with NE$^+$ disease can be linked to specific clones, despite there being no genetic alterations driving the acquisition of a NE phenotype. This suggests that clonal and epigenetic evolution go hand and hand. NE$^+$ and NE$^-$ cells can co-exist in the same microenvironment, suggesting only partial modulation of disease phenotypes by the stroma.

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