P69 Evaluating multicellular contributions to the maintenance of AR activity under conditions of androgen deprivation

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Introduction & Objectives: Androgen receptor (AR), a transcription factor, is a chief driver of prostate cancer. It is the key target to treat metastatic PCa, as most of the mechanisms of resistance to castration are associated with AR signaling pathway.

The tumor microenvironment plays an indispensable role in driving the mechanisms that lead to prostate cancer progression and metastasis. This however also contributes to molecular and cellular heterogeneity in deciphering the driving biology for progression. Chromatin landscape plays an important role in influencing AR activity and gene regulation. Methodologically, it is known by ChIP-seq that the landscape of androgen receptor binding sites are altered in lethal end-stage disease and overexpression of androgen receptor helps to drive chromatin opening through FAIRE/ ATAC-seq. Mechanisms driving changes in the chromatin landscape are not only confined to epithelial cells but include surrounding cells as well.

To tease apart the contribution of distinct cell populations to the maintenance of AR activity, we have evaluated a range of co-culture conditions (LNCaP cells with and without THP-1 and CAF's) for the growth of cancer cells *invitro* in presence of agonists and in response to treatment, to represent clinical human prostate for active AR binding sites. We report genome-wide mapping of AR, chromatin remodeling and gene expression profile using various omics approaches and conclude that in vitro co-culture models reveal active AR binding sites, which may be more clinically relevant.

Materials & Methods: Prostate cancer cells (LNCap) were co-cultured with THP-1 monocytes and CAF (cancer-associated fibroblasts) stromal/ inflammatory cell lines in androgen deprivation media (reflecting ADT) prior to agonist (R1881, DHT) and antagonist treatments for 4 and 24 hrs respectively. Cells were sorted using EPCaM marker for LNCaP cells. RNA was isolated, and sequencing performed. In addition, Chromatin was prepared through either formaldehyde fixing followed by sonication for ChIP or fragmented using tn5 transposase for Omni-ATAC and sequencing performed.

Results: LNCaP cells in co-culture models showed higher expression of androgen receptor and androgen receptor-regulated genes than in monoculture. Co-culture helped to maintain the expression of AR-regulated genes in androgen deprivation conditions and in the presence of AR antagonists. The AR binding profiles, chromatin landscape and the expression profile for each condition have been compared and the number of active binding sites leading to differential gene expression identified.

Conclusions: A co-culture model maintaining AR function in androgen deprivation conditions and in the presence of antagonists, may permit more sensitive mapping of AR binding sites which might help us to find clinically relevant biomarker signature that can be tested in patients and reflects AR activity and gene regulation.