Phenotypic, Genomic, and Clinical Associations of Circulating Tumor Cells (CTCs) Lacking Epithelial Biomarkers in Metastatic Castration Resistant Prostate Cancer (mCRPC)

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Background
- Epithelial plasticity (EP) refers to the loss of the epithelial phenotype and replacement with a novel phenotype, including both the epithelial-mesenchymal transition (EMT) and its reverse process, the mesenchymal-epithelial transition (MET).
- EP is a proposed mechanism of immune evasion, drug resistance, apoptotic resistance, and promotion of metastasis.
- There has been extensive research on CTCs in human subjects based on enrichment of cells expressing EpCAM, which includes preclinical analysis of cells that may be EP.
- Here, the non-enrichment Epic Sciences platform was utilized to identify CTCs in mCRPC patient samples that were phenotypically consistent with EP: CTCs not expressing cytokeratins (CK), but expressing malignant biomarkers such as androgen receptor (AR).

Methods
- 221 mCRPC patient blood samples were collected prior to starting Abiraterone (57), Enzalutamide (90), Apalutamide (3), Docetaxel (23), Cabazitaxel (16), and Paclitaxel (3). Patients were monitored for up to 3 years to assess OS outcomes.
- Samples were processed utilizing the Epic Sciences platform.

Phenotypic Features of CTCs Lacking Cytokeratins

- A. Representative CTCs (upper panel) and CK(-) CTCs (lower panel) CTC Cell Images
  - Cytokeratins (CK) are a family of proteins expressed by epithelial cells
  - The canonical definition of a “CTC” includes CK expression, intact nucleus (NP), and no CD45 signal
  - The EP hypothesis postulates that malignant cells can enter the circulation by downregulating epithelial cytokeratin proteins to become more mesenchymal (MET).

- B. CTCs with Malignant Biomarkers Can Lack Cytokeratins
  - CK(-) CTCs frequent co-expression of AR, nucleoli, and Nuclear speckles
  - CTCs lacked trend for less nucleoli, more nuclear speckles, and lower AR expression

- C. Phenotypic Features of CK(-) and CK(+) CTCs
  - Density plots of CK(-) and CK(+) CTCs

Conclusions
- CTCs in the peripheral blood of mCRPC patients expressing AR and lacking both blood lineage marker CD45 and epithelial marker CK, have similar genomic profiles to CTCs expressing CK and display gross genomic alterations canonically associated with prostate cancer
- CK(-) CTCs are associated with poor OS and can provide independent and additive prognostic value to established prognostic factors: line of therapy, presence of visceral metastases, and pre-therapy PSA; none of these features strongly associate with the presence of CK(-) CTCs.
- The presence of CK(-) CTCs, and the association of these cells with poor OS, are consistent with the Epithelial Plasticity hypothesis.

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