Molecular characterization of circulating tumor cells of patients with neuroendocrine prostate cancer

Himisha Beltran[1], Jessica Louw[3], Rachel Krupa[3], Myriam Kossai[2], Dena Marrinucci[3], Juan Miguel Mosquera[2], David M Nanus[1], Scott T Tagawa[1], Ryan Dittamore[3], Mark A Rubin[2]

Departments of 1. Medicine and 2. Pathology, Weill Cornell Medical College, New York, NY 3. Epic Sciences, Inc. La Jolla, CA

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Background

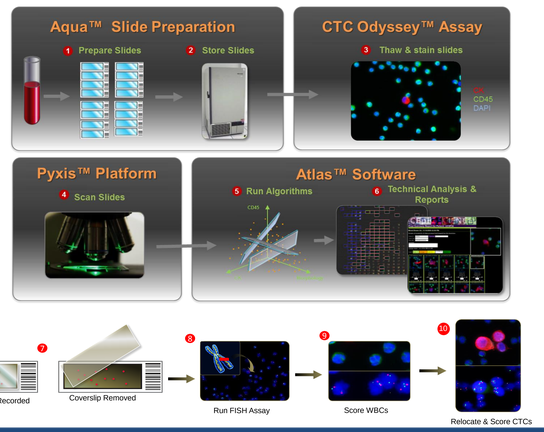
Neuroendocrine prostate cancer (NEPC) is an aggressive androgen independent variant of prostate cancer that most commonly arises in later stages of castration resistant prostate cancer (CRPC) as a mechanism of treatment resistance. Androgen receptor (AR) expression is typically low or absent in NEPC and the genes Aurora kinase A (AURKA) and N-Myc (MYCN) are frequently amplified (Beltran et al, 2011; Mosquera et al, 2013). There are no reliable serum markers to identify patients that are transforming to NEPC and incidence of CTCs in these patients is unknown. Detection of NEPC has clinical implications as these patients would not be expected to respond well to currently approved potent AR targeted therapies for CRPC. Circulating tumor cells (CTCs) are traditionally defined as EpCAM/cytokeratin positive (CK+) cells, CD45-, and morphologically distinct. However, recent evidence suggests that other populations of CTC candidates exist including cells that are EpCAM/cytokeratin negative (CK-) or cells smaller in size than traditional CTCs. CTC positive selection techniques that isolate CTCs based on size, density, or EpCAM positivity may miss CTC subpopulations. We aimed to molecularly characterize CTCs from patients with NEPC utilizing the Epic Sciences platform which performs no physical selection, and to correlate CTC results with patient-matched clinical, molecular, and pathologic features.

Methods

Blood from 11 consecutive patients with metastatic prostate cancer and clinical or pathologic features suggestive of neuroendocrine prostate cancer (NEPC) were collected and shipped to Epic Sciences, where cells were identified utilizing Epic's CTC collection and detection process (Figure 1). Traditional CTCs were identified as CK+CD45- cells with intact DAPI nuclei and after pathologist review of their morphology. Candidate CK- CTC populations were identified as CK-CD45- that were morphologically malignant. Small nuclear size candidate CTCs were identified as CK+CD45- cells with diameters similar to or smaller than that of a typical white blood cells (WBCs). Candidate CTC were evaluated with prostate cancer relevant biomarkers, including androgen receptor (AR) expression by immunofluorescence (IF), a subset of CTCs were stained for EpCAM and Aurora kinase FISH. Exome and RNA-sequencing were performed in patient-matched metastatic biopsies obtained at same time of CTC analysis in 8 cases.

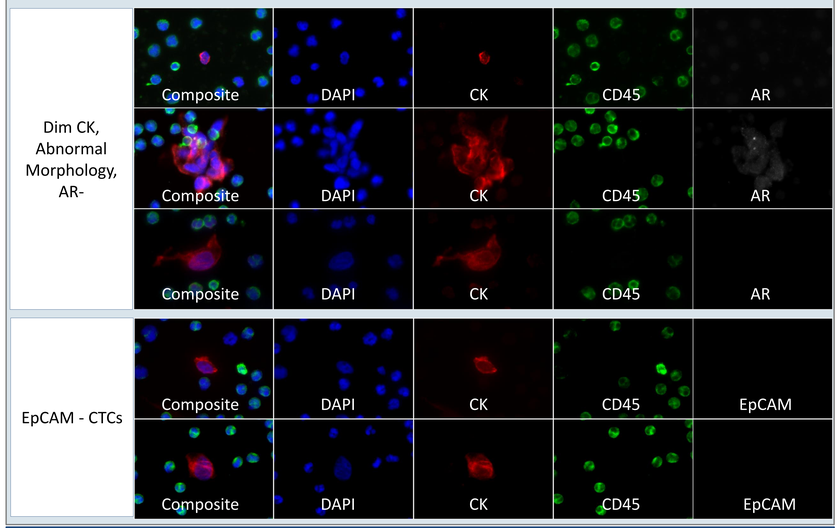
Figure 1: Schematic of Epic's CTC collection and detection process:

1) Nucleated cells from blood sample placed onto slides
2) Slides stored in -80C biorepository
3) Slides stained with CK, CD45, DAPI and AR (or EpCAM)
4) Slides scanned
5) Multi-parametric digital pathology algorithms run
6) Software and human reader confirmation of CTCs & quantitation of biomarker expression
7) For FISH, Coordinates are recorded and coverslip removed
8) FISH assay is run
9) Regional WBCs are scored to assess normal
10) CTCs relocated and scored



Identification of novel CTC subpopulations in NEPC

Figure 2. Low CK CTCs with cancer related morphology are seen which have no AR and no EpCAM expression. CTCs from NEPC patients also exhibit unique spindle-like morphology.



AuroraK amplification seen in tissue & CTC samples

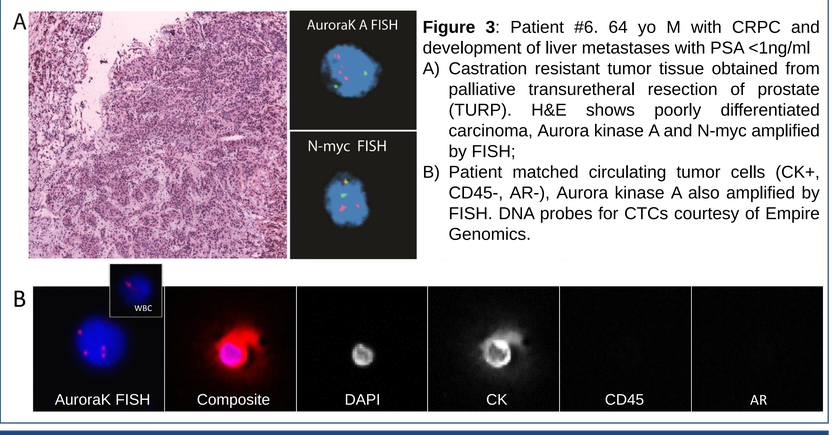


Figure 3: Patient #6. 64 yo M with CRPC and development of liver metastases with PSA <1ng/ml

A) Castration resistant tumor tissue obtained from palliative transuretheral resection of prostate (TURP). H&E shows poorly differentiated carcinoma, Aurora kinase A and N-myc amplified by FISH;

B) Patient matched circulating tumor cells (CK+, CD45-, AR-), Aurora kinase A also amplified by FISH. DNA probes for CTCs courtesy of Empire Genomics.

Clinical Demographics

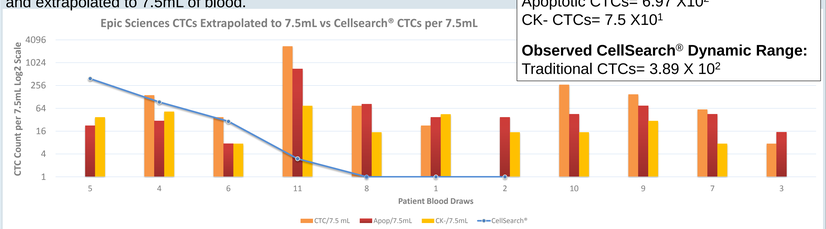
Patients were classified as having pathologically confirmed NEPC arising de novo (n=2) or after treatment (n=4), or clinically diagnosed NEPC based on CRPC with highly aggressive visceral progression and/or PSA <1 ng/ml (n=5). 5 NEPC patients were previously treated with abiraterone and/or enzalutamide. Age range was 62-86 yrs. Sites of metastases included liver (7/11), bone (10/11), lymph nodes (8/11), pleura (1/11), lungs (2/11). Serum PSA, CellSearch® CTCs, and serum NE markers were variable including 5/11 patients with PSA<1 ng/ml (range 0.02-9.39) and 4/6 evaluated pts with CellSearch® CTC count of 0-3 (range 0-94). Metastatic tumor biopsies were evaluated in all cases including one rapid autopsy, with histology ranging from poorly differentiated carcinoma to small cell carcinoma. Neuroendocrine markers (NSE, chromogranin, synaptophysin) showed strong expression by immunohistochemistry in 6/11 cases. 6/8 evaluated tumors displayed amplification of Aurora kinase A by FISH. Patients were subsequently treated with platinum (4/11), AURKA inhibitor (4/11), hormonal therapy (2/11), or progressed to death within 1 day of CTC collection (rapid autopsy case).

Sensitivity of Epic CTC Platform vs. CellSearch®

Figure 4. Matched blood samples were processed utilizing CellSearch® and Epic Sciences CTC platform. Epic CTCs were measured from 1mL and extrapolated to 7.5mL of blood.

Observed Epic Dynamic Range:
Traditional CTCs= 2.78 X10^3
Apoptotic CTCs= 6.97 X10^2
CK- CTCs= 7.5 X10^1

Observed CellSearch® Dynamic Range:
Traditional CTCs= 3.89 X 10^2



AR Expression - Distribution of CTCs

Observed traditional CTCs harbor low or no AR expression

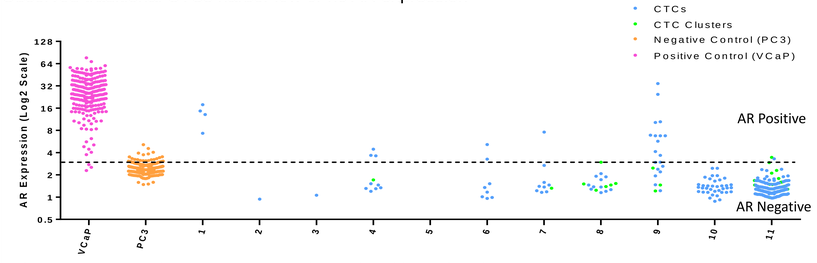


Figure 5. AR expression per CTC or CTC cluster plotted for each patient.

CK Expression - Distribution of CTCs

Observed CK expression on NEPC CTCs was significantly lower than CK expression seen in control cell lines.

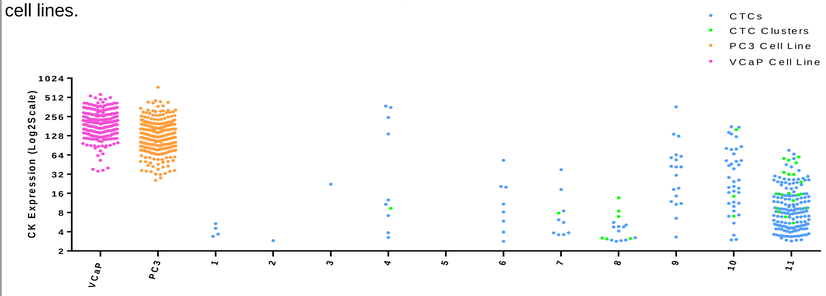


Figure 6. CK expression per CTC or CTC cluster plotted for each patient.

Conclusions

Identification of patients with CRPC progressing towards an AR independent NEPC phenotype remains challenging, especially in the absence of metastatic tumor biopsy. Serum PSA, serum NE markers and CTC count by CellSearch® are unreliable. Epic CTCs from patients with NEPC are smaller in size and show unique spindle like morphology, low cytokeratin expression, and lack AR and EpCAM expression. Epithelial plasticity potentially arising from EMT may explain the lack of detection using conventional CTC assays. We show that the Epic Sciences CTC platform is capable of detecting CTCs from patients with NEPC and CTCs are molecularly similar to metastatic biopsies. Therefore, Epic CTCs may be useful in the earlier detection of NEPC and may potentially inform patient selection for therapy.