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Circulating tumor cells (CTCs) in NSCLC incorporate differential subsets including the epithelial-mesenchymal transition (EMT) phenotype

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BACKGROUND

Non-small cell lung cancer (NSCLC) is a major metastatic tumor for its ability to spread out and generate distant metastases. Recent evidence however suggests that the invasive phenotype of NSCLC is prevalently associated with the epithelial-to-mesenchymal transition (EMT) markers[1]. This study was devoted to improve a DEPArray cell separation protocol by using EMT markers to isolate Circulating Tumor Cells (CTCs) for subsequent molecular analyses by NGS.

RESULTS

Four CD45neg cell subsets were identified in all patients (Fig. 1A), namely: 1) cells expressing only epithelial markers (E-CTC); 2) cells co-expressing both epithelial and mesenchymal markers (EM-CTC); 3) cells expressing only mesenchymal markers (MES-CTC); 4) cells negative for both phenotype markers (NEG-CTC). MES-CTC population was the most represented (58.6%±2.8%) thus supporting the role of EMT in the early phase of tumor spreading (Fig. 1B). Total sequence variants identified by NGS in MES-CTCs were significantly higher than matched FFPE (84.4±41.7 vs 20.4±5.9; p<0.0001) (Fig.2A-B), revealing either inter- or intra-patient heterogeneity. Table 1 shows sequence variants with an allelic frequency threshold upper than 5%.

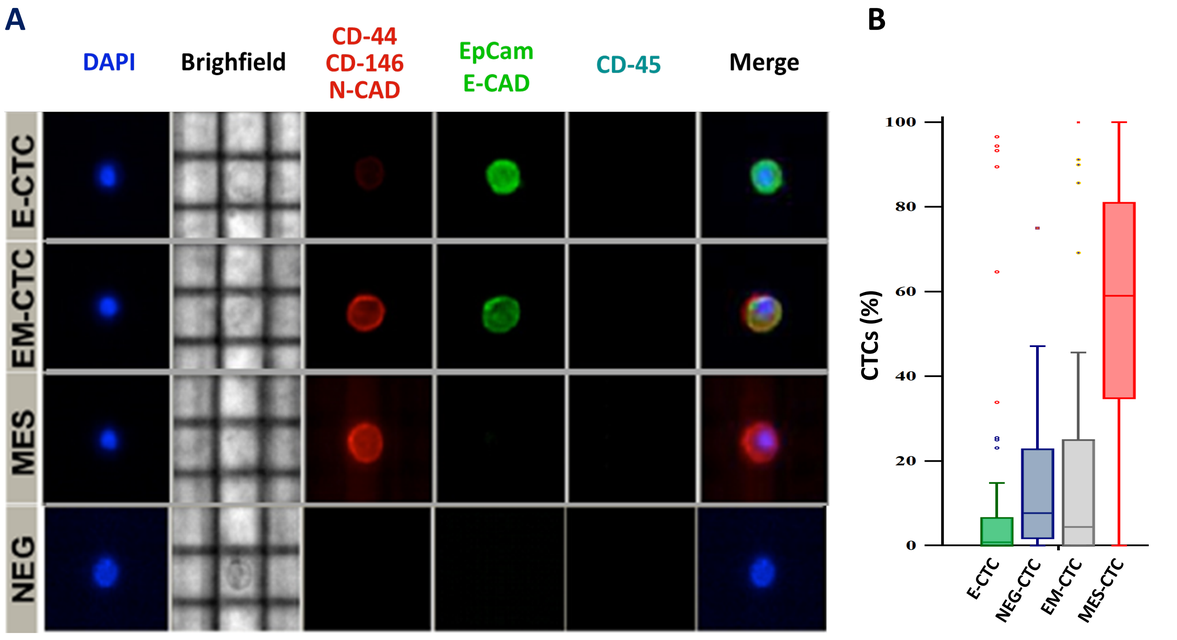

Figure 1. Phenotypic characterization of CTCs and enumeration. *A)* The analysis of EMT markers on CD45neg cells by DEPArray identifies four subsets of CTCs from patients with metastatic NSCLC. *B)* MES-CTCs are prevalent among other CTC phenotypic sub-groups.

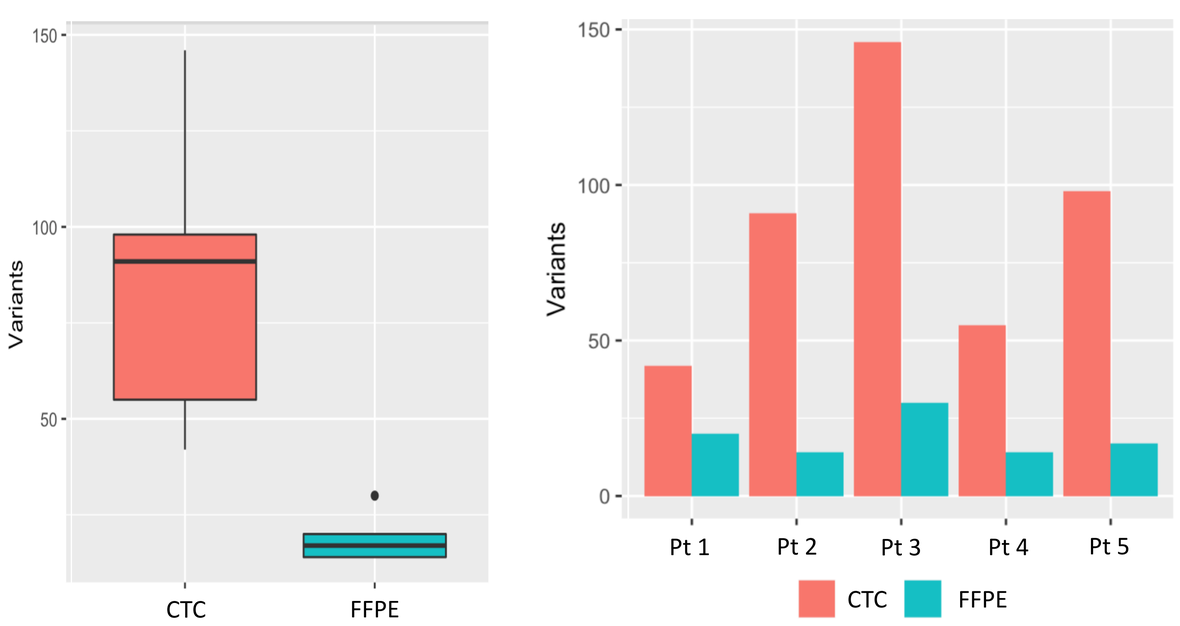

Figure 2. Number of sequence variants identified by NGS in CTCs and matched FFPE samples. *A) Box and whiskers plot* of total variants identified in CTCs and FFPE from 5 patients with NSCLC. B) Bar plot represents inter- and intra-patient heterogeneity relative to all variants identified for each patient in CTCs and FFPE.

MATERIALS AND METHODS

Blood samples from 28 NSCLC patients were depleted of CD45pos leukocytes and stained with an antibody panel to EMT markers directed to EpCAM and E-Chaderin (epithelial) as well as CD-44, CD-146 and N-Chaderin (mesenkymal)[2]. Cell sorting was performed by DEPArray equipment and the recruited CTCs were subjected in a limited number of patients (n=5) to NGS analysis avoiding previous whole genome amplification (WGA)[3] with Ion AmpliSeq™ Cancer Hotspot Panel v2 on the Ion Torrent PGM™ system and compared to FFPE tumor tissue.

Gene	Patient #1		Patient #2		Patient #3		Patient #4		Patient #5	
	FFPE	CTC	FFPE	CTC	FFPE	CTC	FFPE	CTC	FFPE	CTC
APC	c.4479G>A	c.4479G>A	c.4479G>A	c.4479G>A	c.4479G>A	c.4479G>A	c.4479G>A	c.4479G>A	c.4479G>A	c.4479G>A
	wt	wt	wt	c.4744G>C	wt	wt	wt	c.4744G>C	wt	wt
ATM	wt	wt	wt	wt	c.4298C>T	wt	wt	wt	wt	wt
	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt
	wt	wt	wt	c.8066A>G	wt	wt	c.5262G>T	c.5262G>T	wt	wt
	wt	wt	wt	wt	wt	c.5779A>G	wt	wt	wt	wt
	wt	wt	wt	wt	wt	c.5805T>G	wt	wt	wt	wt
	wt	wt	wt	wt	wt	c.5820A>G	wt	wt	wt	wt
	wt	wt	wt	wt	wt	c.5829G>A	wt	wt	wt	wt
	wt	wt	wt	wt	wt	c.5785A>G	wt	wt	wt	wt
ALK	wt	c.3578T>C	wt	wt	wt	wt	wt	wt	wt	wt
	wt	wt	wt	c.3836+7A>G	wt	wt	wt	wt	wt	wt
BRAF	wt	wt	wt	wt	wt	wt	wt	c.1355T>C	wt	wt
CDKN2A	wt	wt	wt	c.347A>G	wt	wt	wt	wt	wt	wt
CSFR1	wt	wt	wt	wt	wt	wt	wt	c.890-7T>C	wt	wt
	c.*35_*36delCAinsTT	c.*35_*36delCAinsTT	wt	wt	c.*35_*36delCAinsTT	wt	c.*35_*36delCAinsTT	c.*35_*36delCAinsTT	wt	c.*35_*36delCAinsTT
EGFR	c.2361G>A	c.2361G>A	c.2361G>A	c.2361G>A	c.2361G>A	c.2361G>A	wt	wt	c.2361G>A	c.2361G>A
	wt	wt	wt	c.2625A>G	wt	wt	wt	wt	wt	wt
	wt	wt	wt	wt	c.2237_2251delAATTAAGAGAAGCAA	wt	c.2237_2251delAATTAAGAGAAGCAA	wt	wt	wt
	wt	wt	wt	wt	wt	wt	wt	wt	wt	c.2262A>G
	wt	wt	wt	wt	wt	wt	wt	c.322A>G	wt	wt
ERBB4	c.421+58A>G	c.421+58A>G	wt	c.421+58A>G	wt	wt	wt	wt	c.421+58A>G	c.421+58A>G
	wt	wt	wt	c.729C>T	wt	wt	wt	wt	wt	wt
	wt	wt	wt	wt	wt	c.2778_2779delGCinsCA	wt	wt	wt	wt
	wt	wt	wt	wt	wt	c.2760C>T	wt	wt	wt	wt
	wt	wt	wt	wt	wt	c.2787C>T	wt	wt	wt	wt
FBXW7	wt	wt	wt	wt	wt	c.1776T>C	wt	wt	wt	wt
	wt	wt	wt	wt	wt	c.1737G>A	wt	wt	wt	wt
	wt	wt	wt	wt	wt	c.1716T>C	wt	wt	wt	wt
FGFR3	c.1953G>A	c.1953G>A	c.1953G>A	c.1953G>A	c.1953G>A	c.1953G>A	c.1953G>A	c.1953G>A	c.1953G>A	c.1953G>A
	wt	wt	wt	wt	wt	wt	wt	wt	c.1959+22G>A	c.1959+22G>A
	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt
FLT3	c.1310-3T>C	c.1310-3T>C	c.1310-3T>C	c.1310-3T>C	c.1310-3T>C	wt	c.1310-3T>C	c.1310-3T>C	c.1310-3T>C	c.1310-3T>C
	c.2053+23A>G	c.2053+23A>G	wt	wt	wt	wt	wt	wt	wt	wt
	wt	wt	wt	c.1323G>A	wt	wt	wt	wt	wt	wt
GNAQ	wt	wt	wt	wt	c.671C>A	wt	wt	wt	wt	wt
HRAS	c.81T>C	c.81T>C	c.81T>C	c.81T>C	wt	wt	wt	wt	c.81T>C	c.81T>C
JACK3	wt	wt	c.2199+17C>T	c.2199+17C>T	wt	wt	wt	wt	wt	wt
KDR	c.3849-24C>A	c.3849-24C>A	wt	wt	wt	wt	wt	wt	wt	wt
	wt	wt	c.1416A>T	c.1416A>T	wt	wt	wt	wt	wt	wt
	wt	wt	wt	c.798+54G>A	wt	c.798+54G>A	wt	wt	c.798+54G>A	c.798+54G>A
	wt	wt	wt	c.799-2A>G	wt	wt	wt	wt	wt	wt
	wt	wt	wt	wt	wt	wt	c.2615-36A>CA	wt	wt	wt
	wt wt	wt	wt	wt	wt	wt	c.798+54G>A	c.798+54G>A	wt	wt
KIT	wt	c.2483A>G	wt	wt	wt	wt	wt	wt	wt	wt
	wt	wt	wt	c.2507T>G	wt	wt	wt	wt	wt	wt
	wt	wt	wt	c.2563T>C	wt	wt	wt	wt	wt	wt
	wt	wt	wt	wt	wt	wt	c.1621A>C	c.1621A>C	wt	wt
	wt	wt	wt	wt	wt	wt	wt	wt	wt	c.136A>G
KRAS	wt	wt	wt	wt	c.37G>T	wt	wt	wt	wt	wt
	c.38G>A	wt	wt	wt	wt	wt	wt	wt	wt	wt
	wt	wt	wt	wt	wt	wt	wt	wt	c.34G>A	wt
MET	wt	wt	wt	c.2518A>G	c.534C>T	c.534C>T	c.534C>T	c.534C>T	wt	wt
	wt	wt	wt	wt	wt	c.2460C>A	wt	wt	wt	wt
	wt	wt	wt	wt	wt	c.2469_2470delCCinsAA	wt	wt	wt	wt
	wt	wt	wt	wt	wt	c.2472_2473delGCinsAG	wt	wt	wt	wt
	wt	wt	wt	wt	wt	c.2454T>C	wt	wt	wt	wt
	wt	wt	wt	wt	wt	c.2484T>C	wt	wt	wt	wt
	wt	wt	wt	wt	wt	c.2487G>A	wt	wt	wt	wt
						c.2534T>A				
NRAS	wt	wt	wt	wt	wt	c.51C>T	wt	wt	wt	wt
PDGFRA	c.1701A>G	c.1701A>G	c.1701A>G	c.1701A>G	c.1701A>G	c.1701A>G	c.1701A>G	c.1701A>G	c.1701A>G	c.1701A>G
	wt	wt	c.2472C>T	c.2472C>T	wt	wt	wt	c.2002+5G>A	wt	wt
	wt	wt	wt	wt	wt	wt	c.1173A>G	c.1173A>G	wt	wt
PIK3CA	wt	wt	c.352+40A>G	c.352+40A>G	wt	wt	wt	wt	c.352+40A>G	c.352+40A>G
	wt	wt	wt	c.1252-35T>C	wt	wt	wt	wt	wt	wt
	wt	wt	wt	c.1252-17T>C	wt	wt	wt	wt	wt	wt
	wt	wt	wt	wt	wt	wt	wt	wt	wt	c.1403A>G
	wt	wt	wt	wt	c.1173A>G	wt	wt	wt	wt	wt
	wt	wt	wt	wt	wt	c.3081C>T	wt	wt	wt	wt
	wt	wt	wt	wt	wt	c.3054C>T	wt	wt	wt	wt
	wt	wt	wt	wt	wt	c.222T>C	wt	wt	wt	wt
	wt	wt	wt	wt	wt	c.171C>T	wt	wt	wt	wt
	wt	wt	wt	wt	wt	c.3141T>C	wt	wt	wt	wt
	wt	wt	wt	wt	wt	c.3266C>G	wt	wt	wt	wt
	wt	wt	wt	wt	wt	c.3282C>T	wt	wt	wt	wt
	wt	wt	wt	wt	wt	c.3245C>T	wt	wt	wt	wt
	wt	wt	wt	wt	wt	c.3270G>A	wt	wt	wt	wt
PTEN	wt	wt	wt	c.717G>A	wt	wt	wt	wt	wt	wt
	wt	wt	wt	c.21G>A	wt	wt	wt	wt	wt	wt
	wt	wt	wt	c.19G>A	wt	wt	wt	wt	wt	wt
RB1	wt	wt	wt	c.2251T>C	wt	wt	wt	wt	wt	wt
	wt	wt	wt	c.1982G>A	wt	wt	wt	wt	wt	wt
RET	c.2307G>T	c.2307G>T	c.2307G>T	c.2307G>T	c.2307G>T	c.2307G>T	c.2307G>T	c.2307G>T	c.2307G>T	c.2307G>T
	wt	wt	wt	wt	wt	wt	wt	wt	c.2712C>G	c.2712C>G
	wt	wt	wt	c.1066C>T	wt	wt	wt	wt	wt	c.1840G>T
SMAD4	wt	wt	wt	wt	c.1060G>C	wt	wt	wt	wt	wt
	c.955+58C>T	c.955+58C>T	wt	wt	wt	wt	wt	wt	wt	wt
	wt	wt	wt	wt	wt	c.1062G>C	wt	wt	wt	wt
	wt	wt	wt	wt	wt	c.1332T>C	wt	wt	wt	wt
	wt	wt	wt	wt	wt	c.1233T>C	wt	wt	wt	wt
	wt	wt	wt	wt	wt	c.1218G>A	wt	wt	wt	wt
	wt	wt	wt	wt	wt	c.1035C>T	wt	wt	wt	wt
	wt	wt	wt	wt	wt	c.1089T>C	wt	wt	wt	wt
	wt	wt	wt	wt	wt	c.1059C>T	wt	wt	wt	wt
	wt	wt	wt	wt	wt	c.990A>G	wt	wt	wt	wt
	wt	wt	wt	wt	wt	c.1002G>A	wt	wt	wt	wt
						c.1353_1359delGGCTACTinsAGCCACG				
	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt
SMO	wt	wt	wt	c.1225G>A	wt	wt	wt	wt	wt	wt
	wt	wt	wt	wt	wt	c.1566G>A	wt	wt	wt	wt
	wt	wt	wt	wt	wt	c.1623G>A	wt	wt	wt	wt
	wt	wt	wt	wt	wt	c.1545T>C	wt	wt	wt	wt
	wt	wt	wt	wt	wt	c.1590_1591delCGinsTT	wt	wt	wt	wt
STK11	wt	wt	wt	c.961C>T	wt	wt	wt	wt	wt	wt
	wt	wt	wt	wt	wt	c.465-51T>C	c.465-51T>C	c.465-51T>C	wt	wt
	wt	wt	wt	wt	wt	wt	wt	c.584T>C	wt	wt
TP53	wt	wt	c.39T>C	wt	wt	wt	wt	wt	wt	wt
	c.215C>G	c.215C>G	c.215C>G	c.215C>G	c.215C>G	c.215C>G	c.215C>G	c.215C>G	c.215C>G	c.215C>G
	wt	wt	wt	c.388C>A	wt	wt	wt	c.388C>A	wt	wt
	wt	wt	wt	wt	wt	wt	c.637C>T	wt	wt	wt
	wt	wt	wt	wt	wt	wt	wt	wt	wt	c.712T>C

Table 1. Comparison of sequence variants between CTCs and matched FFPE. Green: wild type; Yellow: neutral variants; Orange: uncertain pathogenic variants; Red: pathogenic variants. *According to: Cosmic, dbSNP and ClinVar*

CONCLUSIONS

Our data support the suitability of the liquid biopsy in NSCLC patients and confirm the intra-tumor heterogeneity occurring in different patients. Moreover, the classification of CTCs by EMT markers may characterize different CTC subsets that would be lost when using other CTC separation methods including the EPCAM-based recognition by CellSearch technology.

REFERENCE

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