P30 In search of a bone metastasis (BM) gene signature in circulating tumor cells (CTCs) from stage IV breast cancer (BC) patients

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Background

A number of studies have long attempted to define a gene signature of BC osteotropism for the identification of patients to be addressed to BM prevention strategies [1-4]. Based on growing interest for liquid biopsy, we measured in CTCs from metastatic BC patients the expression of major genes functionally involved in BM onset and found a correlation of some gene profiles with metastasis sites.

Methods

Following the local Ethics Committee approval and informed consent, CTCs were obtained from 39 stage IV BC patients, either treatment naïve or in progressive disease, through immunomagnetic pre-enrichment with autoMACS Separator® followed by DEPArray® sorting. CTCs were then assessed by RNAseq in their expression levels of 136 genes, derived from literature, involved in BC progression and BM development. The gene panel was first verified on subclones of the MDA-MB231 BC cell line with different organotropism (P0: parental population; P7: osteotropic subclone; LM: lung-tropic subclone) and then validated in CTCs from patients grouped in relation to their metastatic sites, namely BM-only (BM), other sites (OS), BM and OS (BM+OS).

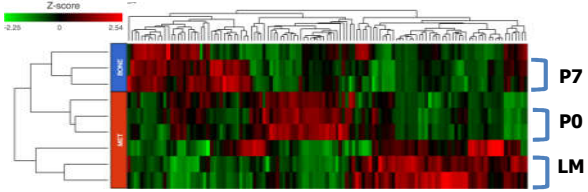

Fig. 1 - BC cell lines with different tissue tropism exhibit distinct GEP.

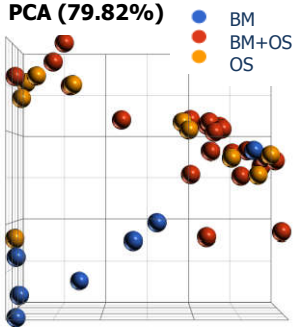

Fig. 2 - CTCs from BM patients clustered separately from other CTCs according to PCA.

Gene	P-value	FDR step up	Fold change
CAPG	1,73E-02	2,02E-01	30,79
HRAS	1,34E-02	2,02E-01	11,67
IL1B	2,46E-02	2,02E-01	5,37
FGFR4	1,99E-02	2,02E-01	5,28
MAF	2,38E-02	2,02E-01	4,39
SERPINB2	2,79E-02	2,02E-01	4,38
CTSK	1,91E-02	2,02E-01	4,06
MAFA	2,77E-02	2,02E-01	3,92
COL3A1	8,53E-03	2,02E-01	3,75
TTYH1	1,37E-03	2,02E-01	3,62
AURKB	3,38E-02	2,08E-01	3,48
HMMR	1,66E-02	2,02E-01	2,97
NAP1L3	2,59E-02	2,02E-01	2,88
EPHB3	3,04E-02	2,02E-01	2,82
SYNM	5,47E-02	2,64E-01	2,74
GIPC1	3,06E-02	2,02E-01	2,43
RERGL	3,03E-02	2,02E-01	2,38
ITGB4	3,38E-02	2,08E-01	2,34
PRDX1	2,23E-02	2,02E-01	2,25
ST3GAL1	8,12E-03	2,02E-01	2,2
MEF2C	3,79E-02	2,24E-01	2,19
DKK1	5,46E-02	2,64E-01	2,18
MAPK1	4,30E-02	2,32E-01	2,17
FGF5	3,01E-02	2,02E-01	2,15
SOX9	4,92E-02	2,57E-01	2,04
FGFR3	1,30E-02	2,02E-01	-2,75
HPRT1	1,24E-02	2,02E-01	-2,79
SMAD2	2,62E-02	2,02E-01	-2,86
HMGA2	2,33E-03	2,02E-01	-4,11
MCM2	3,91E-02	2,24E-01	-4,56
ANLN	9,02E-03	2,02E-01	-12,02

Table 1 DEGs revealed by RNAseq.

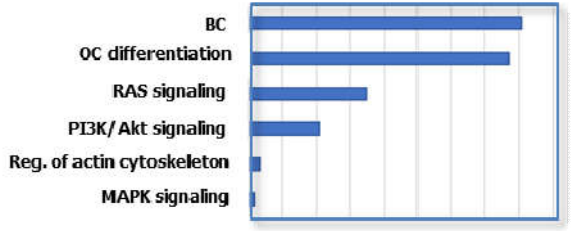

Fig. 3 - Gene Ontology analysis of 31 DEGs emerged from comparison of BM vs OS CTCs.

Results

The transcriptome heatmap of unsupervised hierarchical clustering of BC cell lines, based on normalized read counts, identified distinct profiles in relation to their tissue tropism (Fig.1). The method was then applied to CTCs and the Principal Component Analysis (PCA) demonstrated that CTCs from BM group were separated from both OS and BM+OS groups (Fig. 2). In particular, 31 differentially expressed genes (DEGs) were detected between CTCs from patients in BM versus OS groups (Table 1). By applying Gene Ontology analysis of such DEGs, we found that most of them were functionally enriched in different biological processes enrolled in bone tissue development and morphogenesis, as well as in KEGG pathways (Fig.3).

Conclusions

CTCs isolated from BC patients with different sites of metastases harbor distinct gene expression profile (GEP) that can be successfully evaluated by our assay.

Prospective investigation is desirable to assess the prognostic role of identified DEGs in earlier BC stages.

References

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