RNA-seq analysis: primary breast tumour and circulating tumour cells

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Introduction

In metastatic disease of Breast cancer, cells with origin in the primary tumour are able to migrate to distant organ sites to seed metastatic tumours. These cells, so called circulating tumour cells (CTC) are suspected to be produced more easily by tissues of mesenchymal characteristics. We analysed RNA-sequencing from primary tumour samples of patients with breast cancer. We were looking for changes in gene expressions in primary tumour tissue of the patients with the CTC with mesenchymal characteristics detected in their blood compared to the patients without CTC in blood. To uncover the biological context, the list of differentially expressed genes we gained by analysis was further used for gene enrichment analysis.

Methods

Samples
17 patients - primary tumour samples
8 patients: CTC EMT detected in blood
9 patients: CTC- (not detected in blood)

RNA-seq (Illumina)
2x 75 nt (pair end)

RNA-seq reads analysis pipeline
Raw reads > Trimmomatic > BWA for short reads > FeatureCounts

Statistical comparison
Tool: Deseq2 (R)
Comparison between tumor tissue of patients with mesenchymal CTC (8 patients) & tumor tissue of patients without CTC in blood (9 patients).

Pathway and GO analysis
Tools: g:Profiler

Results

By RNA-seq analysis, we found 70 genes to be up-regulated and 17 to be down-regulated, under the conditions of adjusted p-value<0.1 and fold change>2. These results are shown on the Table 1. Pathway analysis showed down-regulated genes to be often associated with imunology related pathways. Otherwise, differentially expressed genes showed connection to various signaling pathways and interaction between cells. Detailed results are shown on Figure 2.

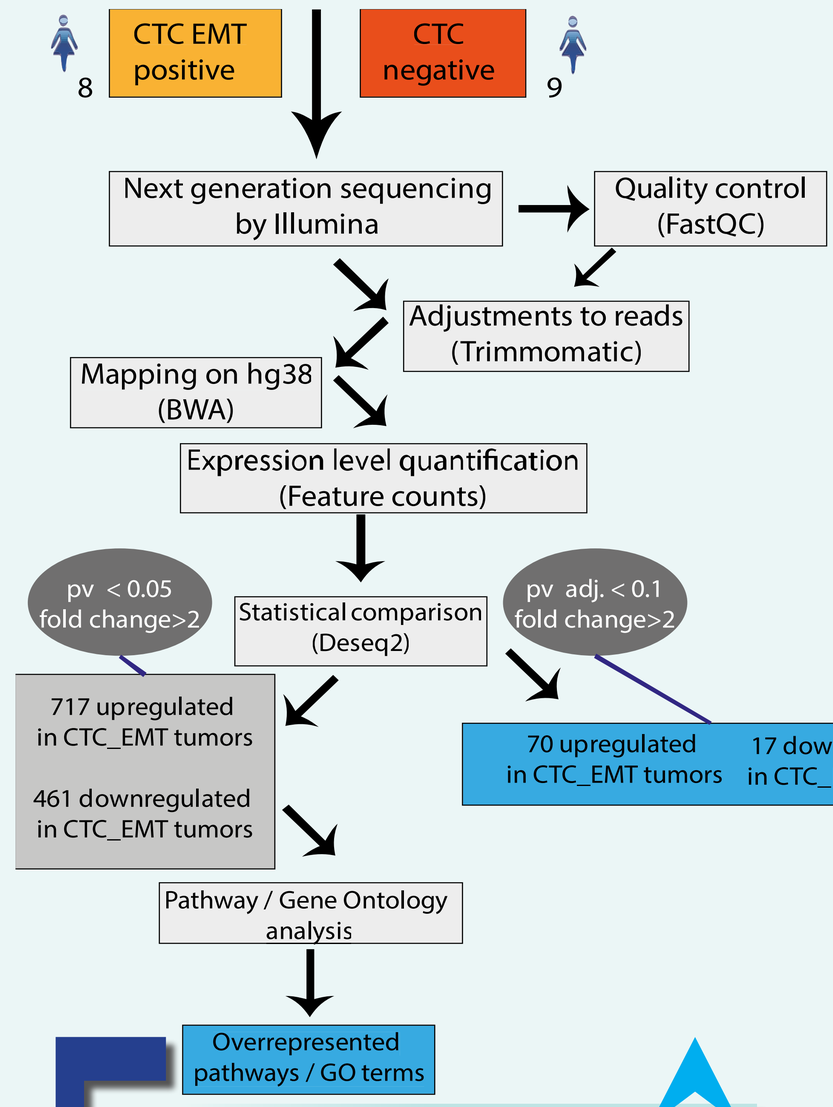

Figure 1: Design of work scheme

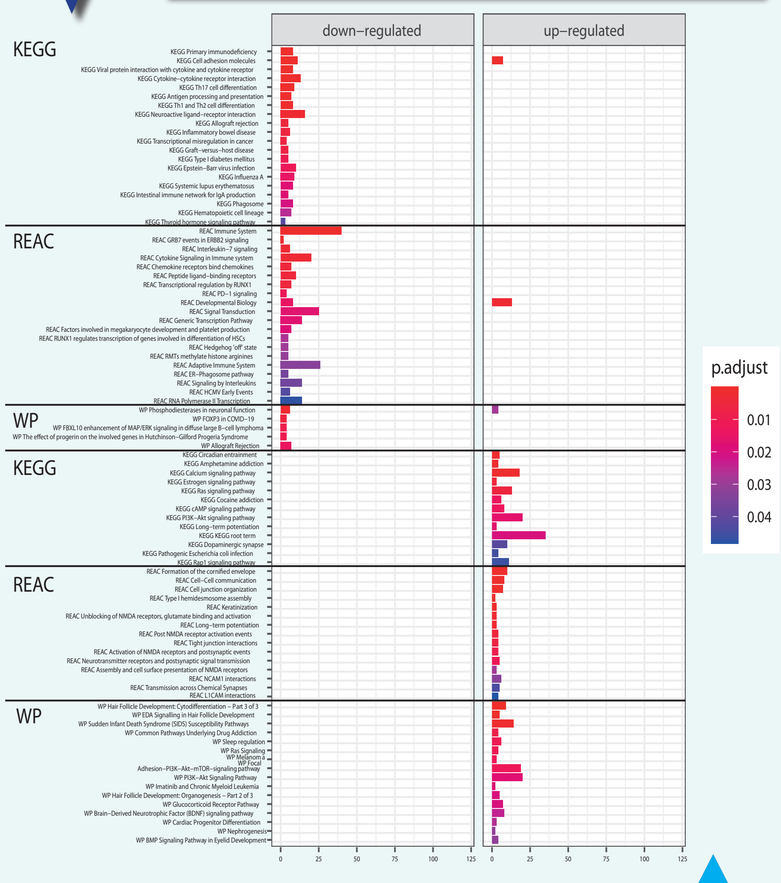

Figure 2: Results of pathways enrichment analysis by g:Profiler.

Up-regulated		Down-regulated
CARTPT	RASD1	LINC01953
KRT5	PNMA8B	TRH
KRT14	INTS4P2	PGAP3
DSC3	ALCAM	MIEN1
ALDH1A2	INTS4	ERBB2
IRX1	MYOZ1	TMEM156
KCTD21-AS1	LOC107986399	PSMD3
KIT	GRIA1	RHOH
KCNE4	TRIM29	STARD3
CALML3	GRIK1	MARK1
TSPAN5	SCN5A	GRB7
ACTN2	CLDN8	MED24
KRT17	TUBB4A	MUC19
TAT	JUN	MYBL2
SCGB3A1	ALG8	UBD
RCAN1	ROCR	IGF1R
C10orf90	HPSE2	FAM83B
FERMT1	PGAP1	
CLDN11	CLNS1A	
GAB2	NCAM1	
GPC3	GDF10	
VEPH1	NLGN1	
FMN2	COL17A1	
GEM	SPHKAP	
LRFN5	PCK1	
LINC02813	LOC107984270	
SMYD1	LOC107984268	
LOC100129434	NDUFC2	
LOC107986984	PAAF1	
ZNF483	ITIH5	
SOX10	CST5	
APOD	OVCH2	
GRIN2A	DUSP1	
OSR1	LOC105371267	
INSYN2B	TP63	

Table 1: Differentially expressed genes in primary tumours. Comparison between tumor tissue of patients with CTC with mesenchymal characteristics & tumor tissue of patients without CTCs in blood (adjusted p value<0.1).

Conclusions

We identified genes with distinct expression profiles in primary tumours of patients conataining CTC with mesenchymal characteristics in their blood compared to patients lacking CTC in their blood. These genes have potential as markers of more aggressive disease, treatment targets (in condition of further validation study). Previous studies of genes show their roles in various cancer related processes. Gene enrichment analysis proposes pathways involved in the processes related to CTC production and subsequent metastasis.
Results of RNA-seq study were further compared by DNA microarray and qRT-PCR. These results (not showed here) will be published soon.
To better understand processes influencing CTC production ongoing in primary tumour, we propose further study by single-cell approach.

Acknowledgements

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Genes

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Abstract:

Circulating tumour cells (CTC) are cells with origin in tumour tissue, which are able to leave their original tissue and travel to distant organs, where they can establish new metastasis. Epithelial to mesenchymal transition (EMT) is believed to influence cells' ability to become CTC. In our research we studied gene expressions in breast tumour and normal breast tissue of breast cancer patients. We investigated which genes expressions are altered for tissue of patients with CTC EMT (positive on mesenchymal markers) in their blood. Gene expressions were obtained by whole transcriptome RNA-sequencing of the fresh frozen primary tumour samples. The study included 18 patients with primary breast cancer and 5 donors of normal breast tissue. We used standard procedure for differentially expressed genes analysis (using tools in Galaxy and R environments) and then used set of differentially expressed genes for gene enrichment analysis in purpose of relevant pathways identification. From RNA-seq analysis, we found 70 genes to be up-regulated and 17 to be down-regulated, under the conditions of adjusted p-value<0.1 and log2FC>1. Downregulated genes were showed to be related to immunology, while up-regulated genes to various signalling pathways and cell-cell interactions. This research was funded by APVV-16-0010 project and the OPII programme as the project PROMEDICOV-19, code ITMS: 313011ATA2, co-financed by the ERDF.