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Transcriptomic analysis of MCF-7 cells treated in acidosis

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Abstract

The cancer microenvironment becomes highly acidic in the early stages of cancer. This phenomenon is caused due to poor vascular perfusion, hypoxia and fermentative glycolysis(1). Cancer cells need to adapt to this environment to survive(2). The underlying mechanisms causing these cells to adapt to this niche while the microenvironment becomes toxic to other normal cells is not properly understood. Using unique culture strategies on breast cancer cells and high-throughput data analysis the underlying mechanism of this adaption was studied at a molecular level with a systematic consideration.

Keywords: Systems biology, RNA sequencing, microRNA, Breast cancer, microenvironment

Introduction

When a tumour starts to form most of these pre-malignant cells are avascular. Thereby, they tend to switch to a more glycolytic metabolism to provide sufficient energy for these cells to thrive(3). This metabolic shift causes the cells microenvironment to become highly acidic due to the production of organic acids. RNA sequencing is a next-generation sequencing (NGS) technology that sequences cDNA in order to provide accurate measurement of levels of transcripts (4). Using such data in the context of a proper biological question alongside systems level analysis could lead to great discoveries in medicine.

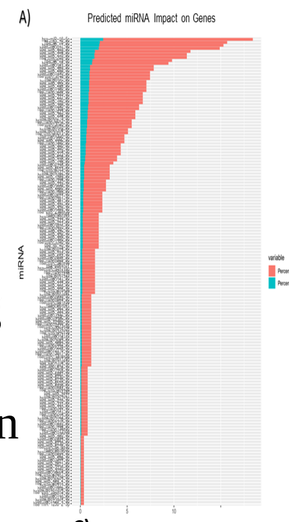
Materials and Methods

RNA sequencing and microRNA sequencing was performed on MCF-7 cells and acid adapted MCF-7 cells. Afterwards, the reads were quality controlled and trimmed using FastQC version 0.11.8 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and Trimmomatic (version 0.39)(3). This was performed to remove low-quality reads and obtain clear reads. Then, these clear reads were aligned to a reference genome using HISAT2 version 2.1.0(4). Finally, these aligned reads were counted to have a quantitative perspective of the mRNAs and microRNAs treated in neutral and acidic pH using HTSeq version 11.1(5). At last differentially expressed genes (DEG) were calculated using R package DESeq2(6). DEGs with the p-value of less than 0.05 were selected to be enriched and analyzed at a systems level to find key pathways or genes related to this adaption. After analysing the raw data n=1928 differentially expressed genes and n=367 differentially expressed miRNAs were explored. Using KEGG, pathways that had been significantly affected by these irregulated genes were enriched. Using validated miRNA database the interactions between differentially expressed RNAs and miRNAs were also explored and analyzed. In order of performing a systematic level analysis the interactions between differentially expressed RNAs and miRNAs were analyzed using miRmapper R package, to identify the most important miRNAs and genes based on their centrality in the interaction network. The hypothesis is that, the miRNA with the largest number of genes to target in a network has the most significant impact on gene regulation towards our phenotype. In this case it shows the most significant miRNAs that are responsible for acid adaption and cancerous cell progression in that harsh environment since they alter a greater proportion of the DEGs.

Results

Predicted miRNA impact on differentially expressed genes based on number of validated interactions and the number of deregulated miRNAs targeting them, prioritized in a decreasing order. The miRNA boxplot (A) and the table (B) are presented in an order of largest number of impacting miRNAs to the lowest. Also, highest ranking genes in terms of interacting with multiple miRNAs were explored and are presented (see table C).

A)



B)

miRNA	Predicted genes found	Percentage of targets	Percentage of DEGs
hsa-miR-16-5p	47	15.98639456	2.437759
hsa-let-7b-5p	40	13.60544218	2.074689
hsa-miR-17-5p	39	13.26530612	2.022822
hsa-miR-92a-3p	38	12.92517007	1.970954
hsa-miR-30a-5p	30	10.20408163	1.556017
hsa-miR-218-5p	29	9.863945578	1.504149
hsa-miR-20a-5p	29	9.863945578	1.504149
hsa-let-7a-5p	25	8.503401361	1.29668
hsa-miR-24-3p	24	8.163265306	1.244813
hsa-miR-34a-5p	20	6.802721088	1.037344
hsa-miR-26a-5p	20	6.802721088	1.037344
hsa-miR-181a-5p	19	6.462585034	0.985477
hsa-miR-19b-3p	19	6.462585034	0.985477
hsa-let-7c-5p	19	6.462585034	0.985477
hsa-miR-125b-5p	18	6.12244898	0.93361
hsa-miR-204-5p	18	6.12244898	0.93361
hsa-miR-30c-5p	18	6.12244898	0.93361
hsa-miR-186-5p	18	6.12244898	0.93361
hsa-miR-221-3p	17	5.782312925	0.881743
hsa-miR-10b-5p	17	5.782312925	0.881743
hsa-miR-10a-5p	17	5.782312925	0.881743
hsa-miR-455-3p	17	5.782312925	0.881743
hsa-miR-29b-3p	16	5.442176871	0.829876
hsa-miR-130a-3p	16	5.442176871	0.829876
hsa-miR-29a-3p	15	5.102040816	0.778008
hsa-miR-215-5p	15	5.102040816	0.778008
hsa-miR-423-5p	15	5.102040816	0.778008

C)

Gene symbol	#	Gene symbol	#	Gene symbol	#
CDK6	25	CREB1	13	CALM1	11
CDKN1B	24	ATM	12	ENTPD1	11
CCND2	21	ACTB	12	CASP3	10
COX6B1	19	CRY2	12	BIRC5	10
CDKN1A	15	CCNG1	11	ZFHX3	10
ATP2A2	14	AHR	11	CSNK2A1	10
CCNF	14	ACVR2B	11	BMP3	10
MAPK14	13	CALM3	11	CDH7	10

To obtain a bright overview of the pathways manipulated by acidification, all DEGs were enriched using KEGG database Top 10 pathways (based on P-value) are shown (see table D).

#Row	Pathway ID	Pathway	P-value
1	hsa05034	Alcoholism	3.1981E-11
2	hsa05322	Systemic lupus erythematosus	1.1957E-10
3	hsa03010	Ribosome	6.1569E-5
4	hsa05203	Viral carcinogenesis	0.0027
5	hsa05416	Viral myocarditis	0.0029
6	hsa05133	Pertussis	0.0034
7	hsa05412	Arrhythmogenic right ventricular cardiomyopathy (ARVC)	0.0037
8	hsa05168	Herpes simplex infection	0.0048
9	hsa05205	Proteoglycans in cancer	0.0076
10	hsa04390	Hippo signaling pathway	0.0087

Discussion, Conclusion and Suggestions

Based on our findings, acidity does significantly affect the transcriptome and other layers of intracellular molecules in order to achieve fitness in the hostile microenvironment surrounding it. Pathways that are responsible for this adaption demonstrate a better vision of how this adaption is achieved. Also, genes such as CDK6 and miRNAs such as hsa-miR-16-5p can be targets to be pursued in further research to get us a step closer to stopping cancer cells before they evolve.

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