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Use microRNA-200c in Endometrial Cancer Treatment with Bioinformatics Tools

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Abstract. Endometrial cancer has two types. In this study we assessed the type 2 by microRNA-200c to find differential genes between the two treatment and control groups and then to find the genes candidate (candidate genes) for microRNA treatment instead of surgery which is the most fundamental treatment in this cancer. By some software's as GEO2R, 85 types (kinds) of genes with differential expression from control and treatment groups were calculated. Also by DAVID tools, the candidates' genes function was extracted. For the first time the genes obtained by microarray data were introduced as candidates for diagnose and treatment of Endometrial cancer cells. The most important genes from the 12 candidates up regulated genes. The 4 genes namely PLAC8, AKR1D1, ANXA3, ESYT2 and from the 9 down regulated candidate genes by miR-200c, 3 genes SLC17A3,THEMIS2, L3HYPDH were reported for the first time in this study. The two above mentioned finding are reported for the first.

Keywords: Treatment, miR-200c, endometrial cancer

1. INTRODUCTION

Every year, endometrial cancer afflicts 14200 women in the world and every year 42000 of afflicted people die. The most samples diagnosed by doctors are after menopause; of course the most dispersion is about 70 years of old. Generally for %80 of afflicted people the survival chance, from diagnosis, is predictable by type 2 is hardly predictable as this type is likely to return even in its first stages. So surgery is the fundamental treatment to this cancer(Amant, Moerman et al. 2005). MicroRNAs are group of non-coding RNAs being 18-25 nucleotides long and after transcription they affect gene expression. They do this by pairing with complement sequence being placed in 3'UTR area related to target RNA(Price and Chen 2014). Some studies report them also in coding sequences and even promoters (Le Quesne and Caldas 2010, Almeida, Reis et al. 2011). The first study, related microRNAs to cancer, was done a lymphocytal leukemia patient. *Kalin* and et observe that in about %50 of these patients, cluster genes (of) miR-150, miR-16 are deregulated or omitted. In fact their repressing tumor role was recommended. Consequently the same group provided the significant percentage of microRNA's map and observed that %52.5 of them fare been placed in connected, breakable and cancer areas. In 2005 the first report on deregulation of 29 of microRNAs in cancer was published (Esquela-Kerscher and Slack 2006, Almeida, Reis et al. 2011). A treatment by microRNA can be done in two ways; the first one by inhibition of microRNA expression and the other one through microRNA substitution, i.e. microRNAs functioning as tumor suppressor and deregulated in cancer tissue are substituted by external microRNA or oncomir action is inhibited in a way. Then based on the way microRNA acts, they can be targeted at two different levels of increasing their area or interference in the reaction between messenger RNA (mRNA)

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and microRNA (Kota and Balasubramanian 2010, Sotillo and Thomas-Tikhonenko 2011). miR-200c is a family of microRNA having various kinds (miR-200a, miR-200b, miR-200c) which are used in this study by taking miR-200c as miR-200c's have an important role in keeping epithelial cells and cause inhibition of EMT (Epithelial to Mesenchymal Transcription) and cell migration and cell movement and also inhibition of proteins having role in ATPase and inhibition of *ZEB1.2*. *E-cadherin* inhibitors have an important role in non-existence of cancer in cells and these important role of miR-200c has differentiated this microRNA from other microRNAs and make it a proper candidate for treatments(Cochrane, Spoelstra et al. 2009, Howe, Cochrane et al. 2011).

2. MATERIAL AND METHODS

A selected experiment was planned to know the genes regulated by miR-200c in aggressive cancer. Then miR-200c was poured into endometrial cancer tissue and diagnose the genes regulated by miR-200c, the treatment sample was examined by normal sample by microarray technique. Microarray data series *GSE25332* was extracted by *NCBI [http://www.ncbi.nim.nih.gov]* and platform: *[HG.U133-plus-2] Affymetrix human genome U133 plus 2.0 GPL570 Array*, from data bank *Gene Expression Omnibus (GEO)*. Three sample of data were divided as a control sample (by 3 repetition), minus control (with (by) 3 repetition) and miR-200c which was our treatment sample.

All were taken from endometrial cancer tissue. To compare control and treatment groups (miR-200c) we omitted minus control sample and compare 3 repetition of miR-200c to control repetitions. The above mentioned data, after extraction to analyze the microarray data to find the genes having differential expression in miR-200c groups compared to control group, were taken to web program *GEO2R (http://www.ncbi.nlm.nih.gov/geo/geo2r)* and by considering the criterion *(Adj.p-value<0.01 and LogFC >1)* consequently after analyzing, it was known that 85 genes have had differential expression and 54 genes up regulated to validate the microarray analysis (Barrett, Wilhite et al. 2013), the genes list in function(operation) tools of the genes having differential expression: 85 genes extracted in database *DAVID (www.david.abcc.ncifert.gov)* went under functional Annotation clustering(Dennis, Sherman et al. 2003, Huang, Sherman et al. 2007, Sherman, Huang da et al. 2007). Analysis by *BioGPS*. The extracted genes by *BioGPS* tools *(www.biogps.org)* were examined by comparing them to another 144 microarray banks to find the place on gene expression in normal samples (Su, Cooke et al. 2002, Wu, Macleod et al. 2013).

3. RESULTS

Comparing of microarray data in the treatment sample (induction of miR-200c into endometrial cancer) and control sample (Endometrial Cancer) to each other leads to find 85 kinds of genes with differential expression that in these review 31 genes were inhibited by miR-200c and 54 kind of genes up regulated by miR-200c (Fig.2).

The 54 genes which up regulated are which the genes *CDH1, ARL14, MAL2, ARHGDIB* and *CDS1* are the first genes which have increased expression (*Log-FC*= 3.55, 188, 182, 1.8 and 1.13, respectively). As an example the gene coding the *CDHI* and having the most expression increase, have had the basic role in cell bindings and keeping the specific shape or differential and somatic cell in its operation place. In most cancers this genes are attacked and inhibited and its control causes The *E-Cadherin* protein not to be produced, and then somatic cells, by losing their bindings, will lose their spatial shape gradually and change into a cancer shape, The results conform to the previous knowledge of cancer (Fig.2).

But the 31 genes attacked and controlled by miR-200c in endometrial cancer, means that these 31 kind of genes have been activated in endometrial cancer and have increased expression,

but after the presence of miR-200c have down regulated, and this decrease was to the amount that has been treated as differential expression, i.e. their *Fold-change score(FC-score)* has been raised equal or less than -1 unit meaning that the expression has been decreased as twice which shows the significance of the transcription measure resulted by genes. From these 31 genes, the five first genes which have experienced the most change are *OMTM1, QKI, ZEB1, HOMX1* and *FN1*, respectively, whose *FC-score* are -1.94, -1.61, -1.64, -1.55, -1.45 respectively. *OMTM1* has down regulated near fourfold, and as it is clear their expression measures are near to each other. (Table.2).

4. BIOGPS ANALYSIS

The gene *OSTM1* codes a protein having a transitional role in *Osteoporosis cells* and in some parts of body as *retina* and epithelial cells of bronchus has high expression in normal condition. Examination of *QKI* in 174 microarray banks shows that this gene has the highs expression in *monocyte* and *Myeloid cells*.

ZEB1 analysis in 174 microarray banks shows that this gene has its highest expression in *uterine cells* and this gene represents firstly the correctness of analyses and secondly the probably important of this gene's role in endometrial cancer (Fig.1).

HMOX1 analysis showed highest expression in this gene in *monocyte, NK cells and endothelial CD105*.

FN1 analysis in 174 microarray data showed highest expression in this gene in *placenta, smooth muscles cells*, the cells producer of *fat*, that its importance in its expression in *smooth muscles* explains its high expression presence in *endometrial cancer cells*.

5. DAVID ANALYSIS

But after functional assess of the two groups of gene (Up regulate-Down regulate) by *DAVID* the following results were obtained. In the first group genes inhibited (controlled) by miR-200c, *DAVID* tool diagnosed 30 genes from 31 genes and in 30 genes no significant common function was found. But in the second group, extracted by microarray analysis sample, from 54 extracted genes, 53 genes were diagnosed by *DAVID* and from 53 genes, 7 genes were found as *cell junction* (Table1).

For the first time, after assess on the up regulated genes, 12 genes of 54 genes, having significant in treatment group have been reported in this study.

It means that these genes have had lower expression or on expression and after induction of miR-200c into treatment group, their expression has increased. From 12 genes not reported in the studies related to miR-200c, causing their increase expression, 6 genes were diagnosed by *BioGPS* analysis and their comparing to 174 microarray banks, which have the same (common) roles as housekeeping genes in all part of body which are *ARL14, TMEM80, TENM2, DOK7, DCAF12L1, SLC25A45* having the roles of *Ribozillation, transition in transitional membrane, connector in protein membrane, relating factor and salt carrier in all part of body*, respectively. But from the other 6 genes, 2 genes *LOC554207, LOC729680* have no role in any bank and no place for expression. From the 4 genes, not reported and influenced by miR-200c causing their increase expression, *PLAC8, AKR1D1, ANXa3, ESYT2* have a high expression in *NK cells, epithelial cells of bronchus, live, embryo liver, dendrite cells* and *burkit's lymphoma*, respectively after comparing to 174 microarray data (Table.2).

In down regulated genes being inhibited by miR-200c, 10 genes were reported for the first time in this study, from which *TUBB4A, DIRAS2* have high expression in all parts of brain. Also the two genes *TLL2, RIPPLY1* have high expression in all parts of body, as housekeeping genes, except for endometrial cancer. The two genes *RHOJ, OAF* having high expression endometrial

cancer show not expression by comparing 174 microarray data. Only the three genes *L3HYPDH, THEMIS, SLC17A3* from the 9 inhibited genes, have high expression in some significant points as *smooth muscle cells, monocytes, myeloid* and *kidney cells* (Table.3).

6. DISCUSSION

The results by this study showed that miR-200c can be a medium for treatment of endometrial cancer. For the high influence of miR-200c on different components of cancers resulted by stem cells or some cancer with stem cells origin, miR-200c by playing role in inhibition of *TGFB, SEMT, ZEB1,2, β-Catenin, BMI1* and having role in various cancers specially those related to stem cells, cause miR-200c treatment to be a hope for endometrial cancer treatment. Many researches have been conducted in this way (Sun, Jiao et al. 2014). Also the findings of this study have reported 9 genes inhibited by miR-200c for the first time. From these 9 genes 3 genes (*L3HYPDH, THEMIS, SLC17A3)* are treated more important than the 6 other genes for their expression in other areas like smooth muscle cell, Monocytes and Myeloid and it is recommended that a *Polymerase Chain Reaction*(PCR) technique is taken from these 9 genes in treatment sample to confirm candidates (Table.3). In this study, for the first time 12 genes are reported as candidates genes that miR-200c has caused their up regulated and it is recommended that, for their final confirm, researchers take *PCR* technique of 12 up regulated genes is treatment samples, and confirm the result of this study (Table.2). From these non-reported candidate genes before this study, these 4 genes *ESTY2, ANXA3, AKR1D1* and *PLAC8* have more important role in endometrial cancer which has high expression in *NK cells, bronchus epithelial cells, liver and uterus liver, Dendrites and Burkitt's lymphoma.* Seven genes from up regulated genes, by *DAVID* Analysis, showed that they have function in *cell conjunction* and from which *DOK1* are the only candidate gene is this study which has up regulated by miR-200c (Table.1).

It is hoped that these candidate genes, in this study, will be used in treatment of endometrial cancer in future. Also there are still some questions like which factors influence miR-200c? And how can understand the exact functionality of miR-200c during cancers treatment, which have not been responded yet. It is hoped that a cure of cancers by microRNA will be obtained someday and to do this more Bioinformatics and clinical studies are required (Iguchi, Kosaka et al. 2010, Andorfer, Necela et al. 2011).

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Table 1. The genes from up regulated genes which are about 54 genes, 7 genes of which have significant function in cell junction diagnosed by *DAVID*.

Gene Name	Gene title
MARVELD2	MARVEL domain containing 2
CDH1	Cadherin 1, type 1, E-cadherin (epithelial)
CGN	Cingulin
DOK7	Docking protein 7
EPB41L5	Erythrocyte membrane protein band 4.1 like 5
PPL	Periplakin
PKP2	Plakophilin

Table 2. 12 genes not reported from 54 genes having increase expression by miR-200c in endometrial cancer.

Gene Name	LogFC
ARL14	-1.88
LOC729680	-1.55
ESYT2	-1.38
TMEM80	-1.25
TENM2	-1.19
ANXA3	-1.16
DOK7	-1.12
LOC554207	-1.07
AKR1D1	-1.06
DCAF12L1	-1.05
PLAC8	-1.02
SLC25A45	-1.01

Table 3. 9 non-reported genes from the 31 genes inhibited by miR-200c in endometrial cancer. Which are reported from the first time in this article.

Gene Name	LogFC
RIPPLY1	1.35
RHOJ	1.3
OAF	1.18
L3HYPDH	1.1
THEMIS2	1.06
SLC17A3	1.06
TLL2	1.02
DIRAS2	1.02
TUBB4A	1.01

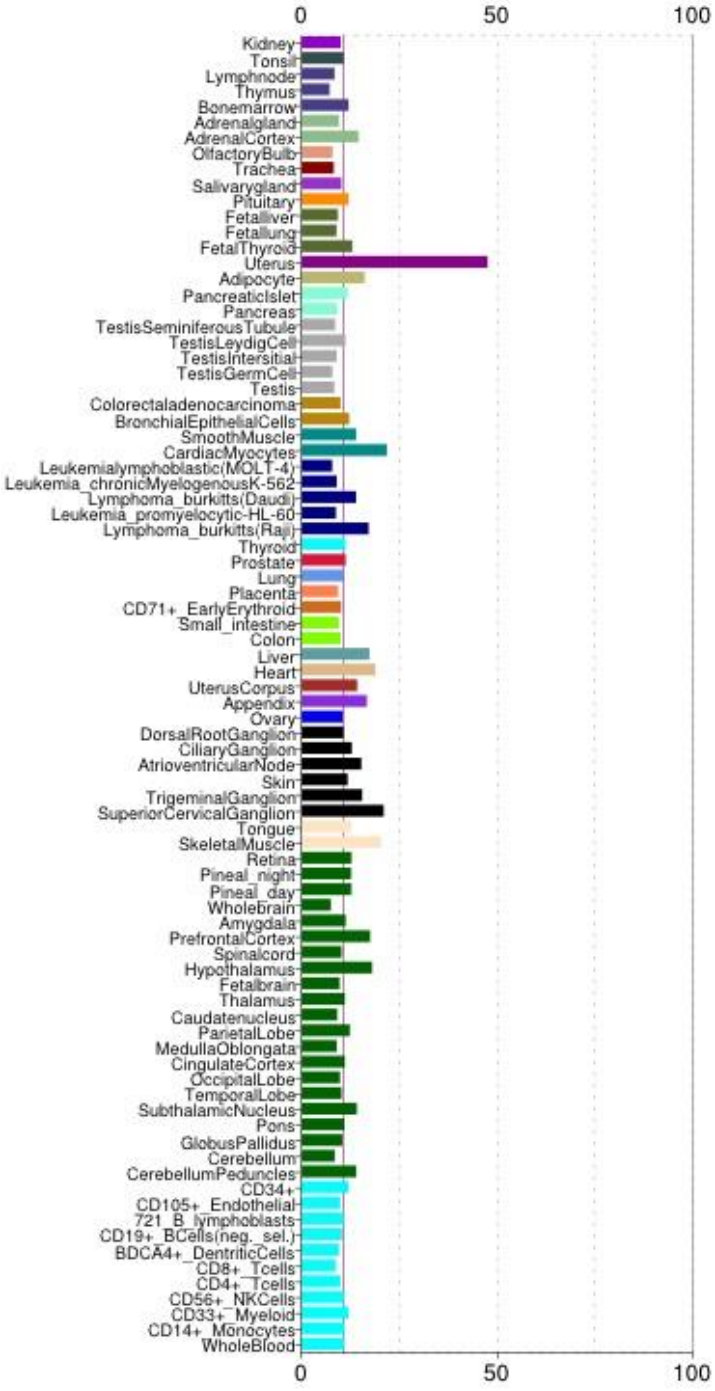

Figure 1. ZEB1 gene has its highest expression in uterine. This analysis has done by BioGPS with comparing to 174 microarray libraries.

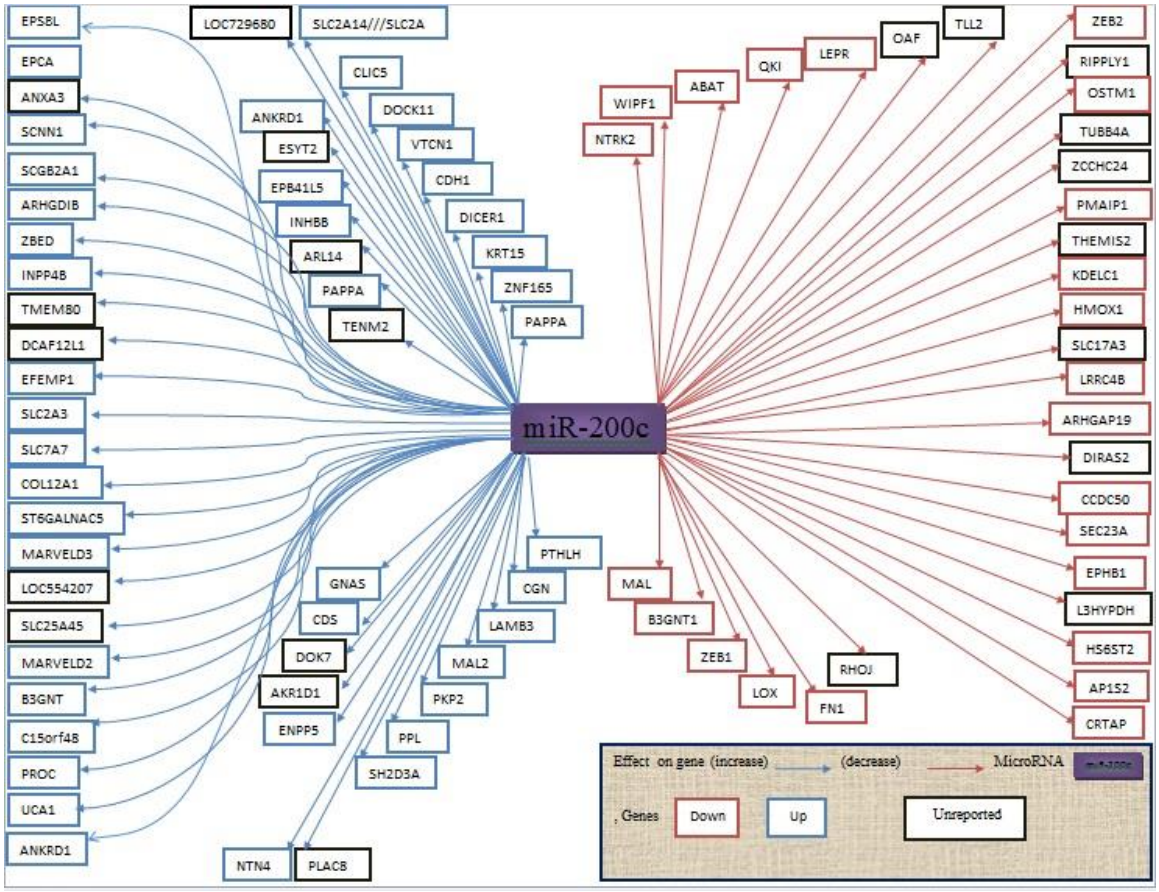

Figure 2. The blue boxes contain 54 kinds of genes having the low expression in control sample but have up regulated in treatment sample. The red boxes contain 31 genes having a high expression in control sample but a low (Decreased) expression in treatment sample inhibited by miR-200c, so their transcription measure has decreased. The black boxes contain the genes not reported in this study and the influence of miR-200c on them has been reported for the first time.