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Housekeeping genes in prostate tumorigenesis

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Abstract

Housekeeping (HK) genes are involved in basic cellular functions and tend to be constitutively expressed across various tissues and conditions. A number of studies have analyzed the value of HK genes as an internal standard for assessing gene expression, but the role of HK genes in cancer development has never been specifically addressed. In this study, we sought to evaluate the expression of HK genes during prostate tumorigenesis. We performed a meta-analysis of gene expression during the transition from normal prostate (NP) to localized prostate cancer (LPC) (*i.e.*, NP>LPC) and from localized to metastatic prostate cancer (MPC) (*i.e.*, LPC>MPC). We found that HK genes are more likely to be differentially expressed during prostate tumorigenesis than is the average gene in the human genome, suggesting that prostate tumorigenesis is driven by modulation of the expression of HK genes. Cell-cycle genes and proliferation markers were up-regulated in both NP>LPC and LPC>MPC transitions. We also found that the genes encoding ribosomal proteins were up-regulated in the NP>LPC and down-regulated in the LPC>MPC transition. The expression of heat shock proteins was up-regulated during the LPC>MPC transition, suggesting that in its advanced stages, prostate tumor is under cellular stress. The results of these analyses suggest that during prostate tumorigenesis, there is a period when the tumor is under cellular stress and therefore may be the most vulnerable and responsive to treatment.

Keywords

prostate cancer; gene expression; meta-analysis; housekeeping genes

Introduction

Although most genes are constitutively expressed in only a subset of tissues, some genes are required for the maintenance of basal cellular functions and tend to be constitutively expressed across various human tissues and conditions. These genes are called housekeeping (HK) genes¹⁻³. Several sets of HK genes have been proposed^{1, 2, 4, 5}.

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Additional Supporting Information may be found in the online version of this article.

Results from a number of studies suggest that modulation of the expression of HK genes is associated with cancer. For example, cytochromes are associated with the risk and progression of pancreatic cancer ^{6, 7}, and translation initiation factors play a role in the development of colonic and esophageal cancers ^{8, 9}. In addition, abnormal expression of ribosomal proteins was reported to be a hallmark of colorectal cancer ¹⁰. Although these and other results suggest that abnormal expression of the HK genes plays an important role in carcinogenesis, a systematic assessment of their role in cancer development had not been yet conducted.

We therefore performed a meta-analysis of publicly available gene expression data to evaluate the expression of HK genes during prostate cancer progression. Our results revealed an association between modulation of expression of HK genes and tumor progression and also suggest that the development of prostate cancer is driven by modulated expression of HK genes.

Material and methods

HK genes

We used 3 lists of HK genes for our meta-analysis. The first list comprises genes showing constant expression in various human tissues ⁴. Because that study was reported in *Trends in Genetics*, we called this set of HK genes TG_HK. The second list was obtained by using a naïve Bayes classifier with specific functional characteristics of HK genes ¹; we called this set of HK genes NB_HK. The third list of genes came from an article by de Jonge *et al.* ². The authors identified a set of 15 genes encoding for ribosomal proteins as the most stably expressed across multiple cell types and different experimental conditions. Thus we called this third set of HK genes 15_HK.

These 3 lists overlap substantially. We found that 124 (22%) of the HK genes were the same in the TG_HK and NB_HK lists. And among the 15 most stably expressed genes from the de Jonge *et al.* paper ², 6 were also reported in the TG_HK list and 14, in the NB_HK list.

Gene expression datasets

The list of the gene expression datasets we used in this study is shown in Table I. Datasets were retrieved from the Oncomine database ¹¹, accessed December 15, 2008. We used 18 datasets in total: 11 for the transition from normal prostate (NP) to localized prostate cancer (LPC) and 7 for the transition from LPC to metastatic prostate cancer (MPC).

Meta-analysis

To estimate the overall statistical significance based on the statistical significance of the individual tests, we used an extension of Stouffer's method ¹². This approach is based on estimating the standard normal deviation, Z . The individual probability, p , is first converted to a Z score, and the Z scores are summed up across studies. This sum is divided by the square root of the number of tests (k). The sum of normal deviates is itself a normal deviate and can be back-transformed into an overall p ; the probability level associated with the sum of Z yields an overall level of significance. The advantage of this method lies in its increased

power: if, for example, several tests consistently favored the research question but failed to reach the level of significance because of small sample size, the overall test would more easily become significant. This approach is similar to the approach recently proposed by Ochsner et al.¹³ The selection of the method of meta-analysis for our study was dictated by the available data: for the majority of the datasets the raw gene expression data were not available, while t-tests and corresponding *p* values were easily accessible for individual probes.

Distribution of the genes by pathways

We used the Database for Annotation, Visualization, and Integrated Discovery (DAVID)¹⁴ to assess the distribution of the differentially expressed genes across the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways^{15, 16}. DAVID tests the null hypothesis that the genes are uniformly distributed across the pathways. The *p* values characterize the statistical evidence for the clustering of the genes by the pathways; the lower the *p* value, the stronger the statistical evidence that the genes tend to cluster in a specific pathway. Student's t-test was used to compare mean expression values between primary tumors with various Gleason scores.

Results

Proportions of the up- and down-regulated genes in the NP>LPC and LPC>MPC transitions

We estimated the proportion of up- and down-regulated genes in the NP>LPC and LPC>MPC transitions. Genes with global *p* values of 10^{-6} were considered to be differentially expressed. We chose this threshold as being a conservative correction for multiple testing with 19,582 as the total number of genes in the NP>LPC transition and 17,902 as the total number in the LPC>MPC transition.

Table II shows the numbers of up- and down-regulated genes. We found that 2,415 (12.3%) of the genes were differentially expressed in the NP>LPC transition. In the LPC>MPC transition, the percentage of differentially expressed genes was lower: 1,574 genes (8.8%). Significant genes in the NP>LPC transition were largely up-regulated—55% of the differentially expressed genes—whereas in the LPC>MPC transition, the significant genes were largely down-regulated—67% of all significant genes.

HK genes are more likely than the average gene in the human genome to be up- or down-regulated

On the basis of what we know about the expression of the HK genes, we expected them to be relatively stably expressed in prostate tumorigenesis. However, the results of our analysis revealed the opposite; the HK genes had a higher proportion of differentially expressed genes than the average gene in the human genome: $173/563 = 0.31$ vs. $2,394/17,859 = 0.13$ ($\chi^2 = 135.1$, $df = 1$, $p \ll 0.001$) for TG_HK genes (Fig. 1). The results were similar for the 3 lists of HK genes. We found that in the NP>LPC transition, HK genes were more likely than the average gene in the human genome to be up-regulated: $122/563 = 0.22$ vs. $1,319/17,859 = 0.07$ ($\chi^2 = 152.5$, $df = 1$, $p \ll 0.001$). In the LPC>MPC transition, HK genes were more

likely to be down-regulated than the average gene in the human genome: $65/563 = 0.12$ vs. $1,052/17,859 = 0.06$ ($\chi^2 = 29.7$, $df = 1$, $p < 0.001$).

Different functional categories of HK genes may have different expression patterns in prostate tumor progression

To look at the expression of the HK genes in more detail, we stratified them into 8 categories: 1) ribosomal proteins, 2) cell communication genes, 3) protein expression (translation) genes, 4) metabolism genes, 5) genes associated with transcription (gene expression), 6) cell structure and motility genes, 7) defense genes, and 8) cell division genes. This stratification was done according to Haverty *et al.*³. (The lists of the genes in each category are in the Supporting Information.)

Table III shows the proportions of up- and down-regulated genes for each category. For most of the functional categories, the expression patterns (*i.e.*, the direction and number of differentially expressed genes) were similar in the NP>LPC and LPC>MPC transitions. HK genes encoding for ribosomal proteins, however, had extremely different patterns of gene expression: in the NP>LPC transition, all ribosomal genes were up-regulated, but in the LPC>MPC transition, all differentially expressed ribosomal genes were down-regulated. We also found that the expression pattern of the genes involved in cell communication differed in the NP>LPC and LPC>MPC transitions. Cell communication genes were preferentially up-regulated in the NP>LPC transition and down-regulated in the LPC>MPC transition.

Gleason score and the expression of ribosomal genes

To pinpoint the time when the expression of ribosomal genes changes from up- to down-regulation, we analyzed their expression in clinically localized cancers with different Gleason scores. We used gene expression data from 3 studies: Luo¹⁷, Singh *et al.*¹⁸, and Welsh *et al.*¹⁹. First, we found that the ribosomal genes are expressed at a higher level than the average gene in the human genome is, which is consistent with their housekeeping role. We also found that expression of the ribosomal genes increased as the Gleason sum score increased from 5 (2 + 3) to 8 (4 + 4) (Fig. 2). The average expression level of the ribosomal genes significantly dropped, however, as the Gleason sum score increased from 8 (4 + 4) to 9 (4 + 5) ($t = 2.7$, $df = 14$, $p = 0.02$), suggesting that the turning point from up- to down-regulation lies between Gleason score 8 (4 + 4) and 9 (4 + 5).

Clustering of the differentially expressed genes in KEGG pathways

According to the changes in their expression during the NP>LPC and LPC>MPC transitions, we subdivided all genes into 8 groups: 1) genes up-regulated in the NP>LPC transition, with no difference in the expression level in the LPC>MPC transition, 2) down-regulated in NP>LPC, with no difference in LPC>MPC, 3) up-regulated in LPC>MPC, with no difference in NP>LPC, 4) down-regulated in LPC>MPC, with no difference in NP>LPC, 5) up-regulated in both NP>LPC and LPC>MPC, 6) up-regulated in NP>LPC and down-regulated in LPC>MPC, 7) down-regulated in NP>LPC and up-regulated in LPC>MPC, and 8) down-regulated in both NP>LPC and LPC>MPC.

We then analyzed the distribution of the genes in each category by KEGG pathways. Table IV shows the results of the analysis with *p* values after Benjamini corrections (DAVID's adjustment on multiple testing)¹⁴. We found that the genes that were significant in only 1 of the transitions (*i.e.*, the first 4 groups) showed no significant clustering by pathway. However, the genes significant in both transitions showed strong clustering. More often than one would expect, the genes up-regulated in both transitions fell in the cell cycle pathway. The cell cycle genes BUB1B, MAD2L1, CDC2, PTTG1, E2F3, CDC6, CCNA2, ORC2L, MCM4, MDM2, CCNE2, GSK3B, CCNB2, BUB1, CDK4, CCNB1, and *PRKDC* include several cycling and cycling-dependent kinases, suggesting that the proliferation rate increases during prostate tumorigenesis. The genes that were down-regulated in both NP>LPC and LPC>MPC transitions were clustered in the focal-adhesion and actin cytoskeleton–regulation pathways. These genes include the integrins and cadherins *ITGA2*, *ITGA5*, *ITGA7*, *ITGA8*, *ITGB1*, *ITGB8*, *CDH3*, and *CDH19*.

Expression of cell proliferation markers

To predict the cell proliferation rate, we estimated the expression of 13 cell proliferation markers: TOP2A, MKI67, PLK1, MCM7, CENPF, MCM6, PCNA, FEN1, MCM2, WT1, CLSPN, CCND1, and *MIB1*. There is published evidence that the expression of all these markers positively correlates with the proliferation rate²⁰⁻³².

We found that in the NP>LPC transition, all of the proliferation markers except *MCM6* were up-regulated, and in the LPC>MPC transition, all proliferation markers, including *MCM6*, were up-regulated (Table V). This suggests that the cell proliferation rate increases during prostate tumor progression.

Heat shock genes

Numerous studies demonstrated that stress changes the expression of multiple genes, with most being down-regulated.^{33, 34} Because gene expression is mostly suppressed in the LPC>MPC transition, one can suppose that the prostate tumor is under stress during this time. We know that increased expression of the heat shock genes indicates cellular stress³⁵⁻³⁷. We therefore assessed the expression of heat shock proteins in the NP>LPC and LPC>MPC transitions. The 34 heat shock genes were retrieved from the NCBI database (Supporting Information). We found that in the NP>LPC transition, 16 heat shock genes were differentially expressed: 9 (56%) were up-regulated, and 7 (44%) were down-regulated. These proportions are very similar to the overall proportions of up- and down-regulated genes in the NP>LPC transition: 55% and 45%, respectively. In the LPC>MPC transition, 14 genes were differentially expressed; 10 of them (71%) were up-regulated. The excess of up-regulated heat shock genes was statistically significant ($\chi^2 = 4.3$, *df* = 1, *p* = 0.01), suggesting that during the transition from localized to metastatic prostate cancer, the tumor cells are under stress.

Discussion

We found that HK genes are more likely than non-HK genes to be differentially expressed in the NP>LPC or LPC>MPC transition (Fig. 1). Because the HK genes are involved in

essential cellular functions, it is unlikely that their differential expression is the result of a ripple effect induced by other genes in driving tumor development. We believe that modulation of the expression of HK genes is a driving force behind prostate tumorigenesis, and it is their differential expression that may induce a ripple effect on multiple downstream targets. This suggests the need to interpret the results of gene expression profiling with caution because the modulated expression of a given gene might be a consequence of the ripple effect induced by other genes rather than being directly related to the phenotype.

We found that the genes tended to be up-regulated in the NP>LPC transition and down-regulated in the LPC>MPC transition. The difference may be driven by overall changes in translation and transcription during prostate tumorigenesis. We also found that ribosomal proteins are up-regulated in the NP>LPC and down-regulated in the LPC>MPC transition: all 30 significant genes were up-regulated in NP>LPC, and all 14 significant genes were down-regulated in the LPC>MPC transition.

Our analysis suggested that the cell proliferation rate increases during prostate tumor progression: the cell proliferation markers were up-regulated in both the NP>LPC and LPC>MPC transitions. We also noted that the genes that were up-regulated in the transitions are clustered at the KEGG cell cycle pathway. Many of the cell cycle genes encoded for cyclins and cyclin-dependent kinases, suggesting that prostate tumor progression is associated with an increased proliferation rate^{38, 39}.

Cell proliferation requires an increased level of transcription and translation, 2 processes that provide building blocks for new cells. In the NP>LPC transition, expression of general transcription factors and ribosomal proteins was increased, with most of the genes differentially expressed in NP>LPC transition being up-regulated. We found, however, that despite the increased proliferation rate, transcription (expression level) and translation (biogenesis of ribosomes) were shut down in the LPC>MPC transition. This is counterintuitive because elevated cell proliferation requires an increase in the biogenesis of ribosomes⁴⁰⁻⁴².

Global suppression of gene expression in the advanced stages of prostate cancer may be a result of the increasing cell proliferation, which surpasses the ability of the tumor vascular system to provide oxygen. We found that expression of 2 of 3 hypoxia-inducible factors, *HIF3A* and *HIF1AN*, was significantly increased in the LPC>MPC transition, with *p* values 0.04 and 0.005, respectively, whereas no difference was found in the NP>LPC transition. Hypoxia and shortages of nutrients and growth factors can induce a stress reaction in advanced prostate tumors. As expected, we found that the expression of stress proteins, which are associated with inhibition of transcription and translation⁴³⁻⁴⁵, is up-regulated in the LPC>MPC transition.

Of note, genes that were down-regulated in the NP>LPC and LPC>MPC transitions clustered into the cell adhesion- and actin cytoskeleton-regulatory pathways. These 2 pathways are mechanistically connected⁴⁶⁻⁴⁸. The reports of a number of studies suggested that modulation of cell adhesion plays an important role in the progression and metastasis of prostate cancer⁴⁹⁻⁵¹.

Figure 3 summarizes our findings on the changes in transcription and/or translation, proliferation rate, and cellular stress during prostate tumorigenesis. On the basis of these findings, one can expect that an advanced tumor is under cellular stress and might be more vulnerable to environmental insults. Since translation is down-regulated at this stage, one can expect that its further suppression can kill tumor cells while causing little or no harm to normal cells with a normal proliferation rate. Currently the available chemotherapies used to treat advanced prostate cancer target DNA replication by introducing chemical links between DNA strands or by blocking DNA replication. Our results suggest that suppression of translation, alone or in combination with suppression of DNA replication, may be beneficial. It is interesting that the results from several studies suggest that inhibition of translation has an anticancer effect. For example, it was demonstrated that the anticancer effect of eicosapentaenoic acid is associated with inhibited initiation of translation⁵². It has also been demonstrated that the anticancer effect of the tumor suppressor Pdc4 is mediated through inhibition of translation⁵³. These findings suggest that inhibition of translation at a time when the tumor is under cellular stress, with already down-regulated transcription and translation, may improve survival and response to treatment.

In conclusion, during prostate tumorigenesis, HK genes are more likely than the average gene in the human genome to be differentially expressed, suggesting that modulation of basic cellular functions drives cancer development. The level of transcription and translation increases during the initial stages of prostate tumorigenesis and decreases during the transition from localized to metastatic prostate cancer. During this stage, prostate cancer is under cellular stress and therefore might be more sensitive to treatment.

Acknowledgments

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Abbreviations

DAVID	Database for Annotation, Visualization, and Integrated Discovery
HK	housekeeping
KEGG	Kyoto Encyclopedia of Genes and Genomes
LPC	localized prostate cancer
MPC	metastatic prostate cancer
NP	normal prostate

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Figure 1.

The proportions of up- and down-regulated housekeeping genes in the normal prostate to localized prostate cancer (NP>LPC) and LPC to metastatic prostate cancer (LPC>MPC) transitions. The red horizontal lines represent the overall proportions of significant genes. Error bars indicate standard error (SE). We used TG_HK genes for this analysis. The results of the analyses of NP_HK and 15_HK genes were very similar.

Figure 2. Dependence of the expression of ribosomal genes on Gleason score. Error bars indicate standard error (SE).

Figure 3. Changes in transcription and/or translation, proliferation rate, and cellular stress during prostate tumorigenesis. Gray bar represents a putative period of increased vulnerability of prostate cancer.

Table I

Datasets used for our meta-analysis

Name	PMID	Sample description (number of samples and metastatic sites [*])	Number of genes
Dhanasekaran_Prostate	11518967	Normal Prostate (22)	9,956
		Primary Prostate Cancer (59)	
Dhanasekaran_Prostate_2	15548588	Normal Adjacent Prostate (12)	19,650
		Prostate Cancer (25)	
Holzbeierlein_Prostate	14695335	Normal Prostate (4)	5,854
		Prostate Cancer (23)	
Lapointe_Prostate	14711987	Normal Prostate (41)	19,116
		Prostate Carcinoma (62)	
Luo_Prostate	11406537	Benign Hyperplasia (9)	6,500
		Prostate Carcinoma (16)	
Nanni_Prostate	16513839	Normal Prostate (3)	22,283
		Prostate Carcinoma (22)	
Tomlins_Prostate	17173048	Benign Prostate (22)	19,355
		Prostate Carcinoma (30)	
Vanaja_Prostate	12873976	Normal Prostate (8)	44,928
		Prostate Adenocarcinoma (27)	
Varambally_Prostate	16286247	Benign Prostate (6)	54,675
		Prostate Carcinoma (7)	
Welsh_Prostate	11507037	Normal Prostate (9)	11,138
		Prostate Carcinoma (25)	
Yu_Prostate	15254046	Normal Prostate (23)	12,625
		Prostate Carcinoma (64)	
Dhanasekaran_Prostate	11518967	Primary Prostate Cancer (59)	9,935
		Metastatic Prostate Cancer (20)	
Dhanasekaran_Prostate_2	15548588	A (1), B (5), LN (5), LG (4), ST (5)	18,502
		Prostate Cancer (25)	
Tomlins_Prostate	17173048	Metastatic Prostate Cancer (6)	19,337
		B (3), LG (3)	
Yu_Prostate	15254046	Prostate Carcinoma (30)	12,625
		Metastatic Prostate Cancer (19)	
Vanaja_Prostate	12873976	B (5), LG (5), LN (4), LI (5)	44,928
		Prostate Carcinoma (64)	
Holzbeierlein_Prostate	14695335	Metastatic Prostate Cancer (25)	6,475
		Distant sites, not specified	
Holzbeierlein_Prostate	14695335	Prostate Adenocarcinoma (27)	6,475
		Metastatic Prostate Cancer (5)	
Holzbeierlein_Prostate	14695335	B (2), LG (1), LN (1), LI (1)	6,475
		Prostate Cancer (23)	
Holzbeierlein_Prostate	14695335	Metastatic Prostate Cancer (9)	6,475
		Metastatic Prostate Cancer (9)	

Name	PMID	Sample description (number of samples and metastatic sites [*])	Number of genes
LaTulippe_Prostate	12154061	B (2), LG (5), ST (2)	12,600
		Prostate Carcinoma (23)	
		Metastatic Prostate Cancer (9)	
		B (4), LG (3), ST (2)	

Pretreatment samples from primary tumors were used for this study.

^{*} A, adrenal; B, bone; LG, lung; LN, lymph node; LI, liver.

Table II

Number of up- and down-regulated genes in the NP>LPC and LPC>MPC transitions *

Transition	Total no. of genes	Significant genes		Total no. of significant genes
		Up-reg.	Down-reg.	
NP>LPC	19,583	1,337 (0.55)	1,078 (0.45)	2,415
LPC>MPC	17,902	520 (0.33)	1 054 (0.67)	1,574

* Parenthetic numbers are the proportions of up- and down-regulated genes.

Table III

Expression of the HK genes stratified by functional categories

Type of HK genes	No. of genes	NP>LPC transition *			LPC>MPC transition *			χ^2	<i>p</i>
		Unchanged	Up-reg.	Down-reg.	Unchanged	Up-reg.	Down-reg.		
Ribosomal protein	59	29 (0.49)	30 (0.51)	0	45 (0.76)	0	14 (0.24)	25.5	1.2×10^{-7}
Cell communication	64	46 (0.72)	12 (0.19)	6 (0.09)	55 (0.86)	0	9 (0.14)	9.2	0.003
Protein expression	37	28 (0.75)	8 (0.22)	1 (0.03)	32 (0.86)	1 (0.03)	4 (0.11)	3.7	0.06
Metabolism	92	67 (0.73)	15 (0.16)	10 (0.11)	72 (0.78)	8 (0.09)	12 (0.13)	1.4	0.24
Gene expression	34	28 (0.82)	5 (0.15)	1 (0.03)	28 (0.82)	1 (0.03)	5 (0.15)	1.1	0.32
Cell structure	33	23 (0.7)	3 (0.09)	7 (0.21)	28 (0.85)	0	5 (0.15)	1	0.31
Defense	35	28 (0.8)	2 (0.06)	5 (0.14)	26 (0.74)	0	9 (0.26)	0.2	0.7
Cell division	14	11 (0.65)	1 (0.14)	2 (0.21)	11 (0.89)	1 (0.07)	2 (0.14)	0	1
Total	368	260	76	32	297	11	60		

* Parenthetic numbers are the proportions.

Table IV

Clustering of genes that are significantly up- or down-regulated in the NP>LPC and LPC>MPC transitions by the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways⁵⁴

NP>LPC *	LPC>MPC *	Significant KEGG pathway
Up (613)		
Down (439)		
	Up (249)	
	Down (292)	
Up (400)	Up (400)	Cell cycle ($p = 3.8 \times 10^{-5}$)
Up (684)	Down (684)	Ribosome ($p = 2.2 \times 10^{-4}$)
Down (266)	Up (266)	
		Focal adhesion ($p = 1.2 \times 10^{-6}$)
Down (657)	Down (657)	Regulation of actin cytoskeleton ($p = 1.6 \times 10^{-4}$)

* Numbers of significant genes are in parentheses.

Table V

Expression of cell proliferation markers during prostate tumor progression

Markers	NP>LPC transition			LPC>MPC transition		
	Z score	Direction	<i>p</i>	Z score	Direction	<i>p</i>
<i>TOP2A</i>	7.09	Up	9.8×10^{-13}	12.19	Up	3.1×10^{-34}
<i>CENPF</i>	5.96	Up	2×10^{-9}	4.54	Up	5.6×10^{-6}
<i>MKI67</i>	4.09	Up	4.3×10^{-5}	7.92	Up	1.9×10^{-15}
<i>MCM7</i>	2.45	Up	0.0142	4.57	Up	4.9×10^{-6}
<i>FEN1</i>	2.12	Up	0.0336	2.16	Up	0.0305
<i>MCM6</i>	-2.01	Down	0.0444	4.09	Up	4.2×10^{-5}
<i>PLK1</i>	1.85	Up	0.0644	7.71	Up	1.1×10^{-14}
<i>PCNA</i>	1.74	Up	0.0816	3.25	Up	0.0012
<i>CCND1</i>	-1.58	Down	0.1132	1.77	Up	0.0771
<i>MCM2</i>	1.55	Up	0.1218	2.06	Up	0.0392
<i>MIB1</i>	-0.91	Down	0.3647	-0.84	Down	0.3983
<i>CLSPN</i>	-0.13	Down	0.8993	-1.96	Down	0.0500
<i>WT1</i>	0.12	Up	0.9059	2.02	Up	0.0434