

RESEARCH ARTICLE

Analysis of Different Activation Statuses of Human Mammary Epithelial Cells from Young and Old Groups

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Abstract

Human mammary epithelial cells have different proliferative statuses and demonstrate a close relationship with age and cell proliferation. Research on this topic could help understand the occurrence, progression and prognosis of breast cancer. In this article, using significance analysis of a microarray algorithm, we analyzed gene expression profiles of human mammary epithelial cells of different proliferative statuses and different age groups. The results showed there were significant differences in gene expression in the same proliferation status between elderly and young groups. Three common differentially expressed genes were found to dynamically change with the proliferation status and to be closely related to tumorigenesis. We also found elderly group had less status-related differential genes from actively proliferating status to intermediate status and more status-related differential genes from intermediate status than the young group. Finally, functional enrichment analyses allowed evaluation of the detailed roles of these differentially-expressed genes in tumor progression.

Keywords: Breast cancer - human mammary epithelial cells - aging - differential genes

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Introduction

Breast cancer is one of the leading causes of cancer-related death among adult females in the world (Jemal et al., 2009). Human mammary epithelial cells (HMEC) model system provides experimentally tractable means to examine the processes involved in normal human cell biology, aging, and carcinogenesis (Tam et al., 2013).

Normal human epithelial cells in culture would encounter a well-characterized genetic and epigenetic barrier- an Rb-mediated stress-related senescence barrier (stasis), and then they would show a limited proliferative potential of 10 to 40 population doublings (Romanov et al., 2001). The status before stasis is called pre-stasis, which has a close relationship with aging and cell proliferation. According to the activation degree of proliferation, pre-stasis mammary epithelial cells are divided into three statuses: actively proliferating pre-stasis HMEC (status 1), intermediate proliferating pre-stasis HMEC (status 2) and pre-stasis HMEC at stasis (status 3). Changes to the balance of pre-stasis mammary epithelial cell lineages in the epithelium may presage susceptibility to breast cancer (Yu et al., 2013). And previous study has found incidence of breast cancer has significant age difference (Eivazi-Ziaei et al., 2011). So it was imperative to understand and master the etiological relationship between changes in the progenitors of normal mammary epithelia and cancer

progression with the increase of age. The research on pre-stasis mammary epithelial cells could also contribute to examine gene expression changes during the process of transformation of normal finite cells to immortality and malignancy.

In this article, we investigated this topic and analyzed the gene expression profiling of pre-stasis mammary epithelial cells in vitro. Using significance analysis of microarrays (SAM) algorithms, we identified differentially expressed genes for different proliferative statuses and different age groups. Furthermore, functional analyses and literature retrievals were used to decipher and evaluate the detailed roles of these differential genes in tumor progression.

Materials and Methods

Gene expression data

Expression profiles were obtained from the Gene Expression Omnibus (GEO) database (<http://www.ncbi.nlm.nih.gov/geo/>). We normalized and estimated the expression for each sample and gene for the two expression profiles separately. After transforming Affymetrix Probe Set ID into gene symbol, we calculated the mean value of the same gene's expression values for each sample if there were duplicate records, and then obtained relative gene expression scores.

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The accession number of morphologically normal human mammary epithelial cell dataset was GSE37485, which contained a large collection of normal human mammary epithelial cell strains from 52 women aged from 16 to 91 years, derived from primary tissues. The accession number of breast cancer gene expression profile was GSE15852, which contained 43 breast tumors and their paired normal controls.

Gene expression significance analysis

Significance analysis of microarrays (SAM) algorithms was used to identify differentially expressed gene sets (Tusher et al., 2001). Genes identified by SAM were filtered by fold change (≥ 2.0). Age-related differentially expressed genes were identified between elderly group and young group in each of the three pre-stasis mammary epithelial cell statuses respectively. Status-related differentially expressed genes were identified between different pre-stasis mammary epithelial cell statuses in elderly group and young group respectively. We also identified differentially expressed genes between breast cancer gene expression profile and normal gene expression profile in elderly group and young group respectively.

Results and Discussion

Age-related differentially expressed genes analysis

For better researching the relation between age and breast cancer development, we divided gene expression profile GSE37485 into different proliferating status groups according to the activation degree of proliferation of pre-stasis mammary epithelial cells, which were divided into three statuses: actively proliferating pre-stasis HMEC (status 1), intermediate proliferating pre-stasis HMEC (status 2) and pre-stasis HMEC at stasis (status 3). Status 1 contained 22 samples, status 2 contained 10 samples and status 3 contained 18 samples (the total number of samples were 52, 2 samples unmatched were removed). Simultaneously, we also divided this gene expression profile into elderly group and young group according to samples aged over 60 and under 45. Elderly group contained 19 samples and young group contained 31 samples. Thus there were 7 samples in status 1, 4 samples in status 2 and 8 samples in status 3 for elderly group and there were 15 samples in status 1, 6 samples in status 2 and 10 samples in status 3 for young group.

Using SAM, age-related differentially expressed genes were identified between elderly group and young group in different pre-stasis mammary epithelial cell statuses, and for each of the three pre-stasis mammary epithelial cell statuses the number of age-related differentially expressed genes were 600, 982 and 1292 respectively. The number of common age-related differential genes in the three statuses was 143.

Gene ontology (GO) enrichment analysis and KEGG annotation analysis were performed for 143 common differential genes using DAVID database (Huang da et al., 2009). The results showed that these differential genes had significantly annotated GO information, such as cell cycle regulation, positive regulation of programmed cell death and cell apoptosis and so on. The formation of

Table 1. The Number of Age-Related Differentially Expressed Genes

	Status 1	Stuatus 2	Status 3
SAM	600	982	1292
regression analysis	672	1008	1128
SAM \cap regression	322	461	572

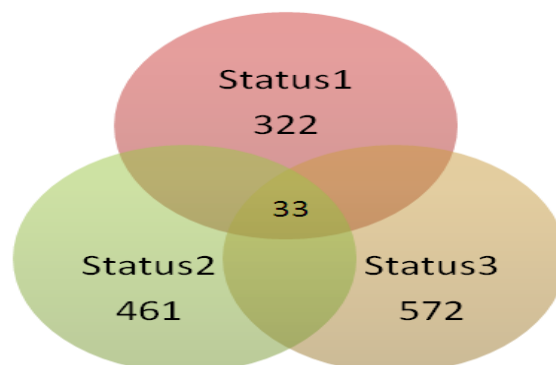


Figure 1. The Number of Age-Related Differential Genes in the Three Statuses

the tumor results from cell proliferation, apoptosis and differentiation. Cell cycle regulation plays a key role in controlling cell function and changing cell proliferation status. Abnormal cell cycle checkpoint could lead to genetic change, and may result in the malignant cell transformation (Xing et al., 2013). Differentially expressed gene PSMA7 has been demonstrated to interact with some important proteins involved in cell cycle transition and even tumor initiation and progression by previous study (Du et al., 2009). Differentially expressed gene CDK5 (Neuronal cell specific cyclin-dependent kinase 5) also plays an important role in the proliferation of breast cancer cells and is functionally involved in cell death pathways induced by anti-cancer drug (Upadhyay et al., 2008).

For further verifying the correlation between age and age-related differentially expressed genes for a given cell proliferating status, we also identified age-related genes with polynomial regression analysis (Significant threshold < 0.001) by R (<http://www.r-project.org>). The results showed the number of age-related genes were 672, 1008 and 1128 for each of the three pre-stasis mammary epithelial cell statuses respectively. The number of the intersection genes from both SAM method and regression analysis method were listed in Table 1. The number of common age-related differential genes from both methods in the three statuses was 33 (Figure 1).

According to literature verification, these common age-related differential genes were related with senescence. With the increase of age, cell cycle and proliferation-related genes expressed unusually. An age-dependent, estrogen receptor-independent gene expression signature identified in type-matched breast tumors, has suggested genetic and/or micro environmental changes which were involved in the pathogenesis of age-related breast cancers (Yau et al., 2007). Common age-related differential gene MAD1L1 is a type of mitotic arrest-deficient proteins, which shows typical morphologies of aggressively proliferating mitotic cells. Yoichi Iwanage et al's results indicated MAD1L1 influences tumor development and

Table 2. The Number of Status-Related Differential Genes in Different Age Groups

	Elderly group	Young group
Status 1-status 2	38	1181
Status 2-status 3	2191	1580

Table 3. The Number of Intersection Differential Genes for Elderly Group and Young Group

	Elderly group	Young group
tumor \cap (Status 1-status 2)*	8	43
tumor \cap (Status 2-status 3) [#]	268	46

**the intersection between tumor-related differentially expressed genes in GSE15853 and status-related differentially expressed genes between status 1 and status 2 in GSE37485, [#]the intersection between tumor-related differentially expressed genes in GSE15853 and status-related differentially expressed genes between status 2 and status 3 in GSE37485

reveals a statistically significant age difference in tumor incidence (Iwanaga et al., 2007). And cell division cycle gene CDC37 has been widely verified to correlate with a hallmark of immune senescence, which is called chronic inflammation or “inflamm-aging” (Didier et al., 2012). Together, our findings provided a cellular basis to explain the observed vulnerability to breast cancer that increased with age.

Status-related differentially expressed genes analysis

For understanding the influence of different pre-stasis mammary epithelial cell proliferative statuses on breast cancer in vitro culture, status-related differentially expressed genes were identified. Taking age into consider, we identified these status-related differentially expressed genes by SAM in elderly group and young group respectively.

Status-related differentially expressed genes in different age groups

In elderly group, the number of differentially expressed genes between actively proliferating pre-stasis HMEC (status 1) and intermediate proliferating pre-stasis HMEC (status 2) was 38. The number of differentially expressed genes between intermediate proliferating pre-stasis HMEC (status 2) and pre-stasis HMEC at stasis (status 3) was 2191. In young group, the number of differentially expressed genes between actively proliferating pre-stasis HMEC (status 1) and intermediate proliferating pre-stasis HMEC (status 2) was 1181. The number of differentially expressed genes between intermediate proliferating pre-stasis HMEC (status 2) and pre-stasis HMEC at stasis (status 3) was 1580 (Table 2).

We could see the number of differentially expressed genes between status 1 and status 2 in elderly group was less than that in young group, but the number of differentially expressed genes between status 2 and status 3 in elderly group was more than that in young group. It suggested that in actively proliferating status, young individuals were more sensitive to the transformation of cell status, had the stronger ability of cell stress, and could answer the stimulation from intracellular and external environment more easily. However, the status from

Table 4. GO/KEGG Functional Enrichment Information for Young Group

	status1-2 \cap tumor	status2-3 \cap tumor
Pathways in cancer	9	
Regulation of cell cycle	12	
Regulation of cell proliferation	30	20
Cellular development process	20	16
Cell differentiation	16	13
Aging		7
Fatty acid metabolic process		11

intermediate proliferating pre-stasis HMEC to pre-stasis HMEC at stasis was the process of becoming blocking, elderly individuals had more blockade-related genes and could more easily break through block and get close to the infinite proliferation of tumor. In addition, the number of differentially expressed genes between status 2 and status 3 was more than that between status 1 and status 2 for both elderly and young group. It accorded with the process of transformation of normal finite cells to immortality and malignancy after encountering the well-characterized genetic and epigenetic barrier.

Status-related differentially expressed genes existed in three proliferating statuses

Taking no account of the influence of aging, we found three differentially expressed genes (FUCA1, FOLR3, PLS1) which existed in all the three proliferating statuses by getting intersection of status-related differentially expressed genes between any two different statuses. According to literature verification, the three genes were significantly related with breast cancer progression. Such as quantitative polymerase chain reaction analysis showed mRNA levels of folate receptor-gamma gene (FOLR3), which plays an important role in mediating folate into the cell, is directly interrelated with ovarian carcinoma and breast carcinomas (Yuan et al., 2009). The high expression of folate receptors in breast carcinoma supported the validity as molecular therapeutic targets in this disease.

The relation between Status-related differentially expressed genes and tumor-related differentially expressed genes

We divided tumor and normal samples in GSE15853 into elderly group and young group, according to the samples aged over 45 and under 45. The number of tumor samples for elderly group and young group were 30 and 13 respectively. And so were the normal samples. By SAM, the number of tumor-related differentially expressed genes was 1274 in elderly group and 388 in young group. We got the intersection between tumor-related differentially expressed genes in GSE15853 and status-related differentially expressed genes in GSE37485 in elderly group and in young group respectively (Table 3).

Gene ontology (GO) enrichment analysis and KEGG annotation analysis were performed for every type of the intersection differential genes, and the results showed significantly annotated information (Table 4 and Table 5). For example, in young group, genes existed in both status 1 and status 2-related differential genes and tumor-related

Table 5. GO/KEGG Functional Enrichment Information for Elderly Group

	status1-2∩tumor	status2-3∩tumor
Cell cycle pathway		15
P53 signaling pathway		16
Regulation of cell proliferation	12	34
Cellular development process	16	26
Protein metabolic process	12	24
Cellular response to stress	12	
Cellular response to stimulus	12	11
Signal transduction	11	74
Biological regulation	8	30

differential genes significantly annotated regulation of cell proliferation, cell differentiation and pathways in cancer. In elderly group, genes existed in both status 2 and status 3-related differential genes and tumor-related differential genes could organize multiple biological pathways, such as cell cycle pathway and p53 signaling pathway. P53 has been shown to play a key role in mediating cell responses to stress (Yang et al., 2012). It is also very well documented that wild type p53 induces apoptosis- one of the hallmark of cancer. P53 primarily accomplishes this by inducing or repressing a number of genes involved in cell cycle arrest, senescence, apoptosis, DNA repair and angiogenesis (Patel et al., 2012).

In conclusion, we analyzed the gene expression profiling of pre-stasis mammary epithelial cells in vitro and identified differentially expressed genes for different proliferative statuses and different age groups with significance analysis of microarrays (SAM) algorithms. Then a mess of functional annotations and literature retrievals were used to decipher and evaluate the detailed roles of these differential genes in mammary cell proliferation and tumor progression. From the results, we saw that there were significant differences in gene expression in the same proliferation status between elderly and young group. Furthermore, elderly group had less status-related differential genes from actively proliferating status to intermediate proliferating pre-stasis status and more status-related differential genes from intermediate proliferating pre-stasis status to pre-stasis HMEC at stasis than young group. These suggested that our status-related and age-related differential genes could succeed in understanding the valuable relationship between changes in the progenitors of normal mammary epithelia and cancer progression. What's more, three common differentially expressed genes were found to dynamically change in different proliferation statuses and were closely related to tumorigenesis. With further experiments providing more complete knowledge of pre-stasis HMEC, more attention should be paid to these important genes in the future.

Acknowledgements

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