

## RUNX genes expressions in breast cancer and fibroadenoma

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### Abstract

**Aim:** Many genes have been identified to control cell proliferation in human such as RUNX gene family. Mutations in these genes have been shown to be responsible for various cancer developments. Our aim in this study is to investigate RUNX gene family expressions in breast cancer and breast fibroadenomas. **Material and Method:** All consecutive patients whose histopathological examination resulted in breast cancer, fibroadenoma, or normal breast tissue between the years 2012 and 2014 were included in the study. Total RNA from each sample was isolated with genomic RNA extraction sample and gene expressions of RUNX1, RUNX2, and RUNX3 were measured with real-time polymerase chain reaction. Gene expressions and patients' characteristics were evaluated among histopathological groups. **Results:** According to statistical analysis, RUNX1 and RUNX2 expressions were upregulated in fibroadenoma patients while only RUNX2 expression was found to be upregulated in breast cancer patients. RUNX1 was upregulated in patients with p53 mutation, whereas RUNX2 was upregulated in patients without p53 mutation. **Discussion:** To the best of our knowledge, our study is the first study that evaluates RUNX1, RUNX2, and RUNX3 gene expressions in both benign and malignant breast disease. RUNX2 gene was significantly upregulated in patients with both breast cancer and fibroadenoma in our study. In contrast, however, upregulated, RUNX1 and RUNX3 gene upregulations in the breast cancer and fibroadenoma patients were not statistically significant. In our study, RUNX2 may be a good prognostic factor in contrast to its role in osteosarcoma or bone metastasis in breast and prostate cancer.

### Keywords

Breast Cancer; Fibroadenoma; RUNX1 Gene; RUNX2 Gene; RUNX3 Gene

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**Introduction**

Breast cancer is still the worldwide most common cancer in females and its incidence increases with advancing age. Screening programs made it possible to determine the disease at an early stage, so that mortality and morbidity decreased [1].

Many genes have been identified to control cell proliferation in human such as RUNX gene family. RUNX1, RUNX2, and RUNX3 are the members of this gene family and they play important roles in biological processes such as regulation of hematopoiesis, bone formation, gastrointestinal and neuronal development. Mutations in these genes have been shown to be responsible for various cancer developments [2].

On the other hand, fibroadenomas are the most common benign breast disease of females that arise from the epithelium and stroma of the breast. It usually develops in young females with a painless, firm solitary mass in the breast. Cytogenetic abnormalities like translocations were found 20-30% in fibroadenomas, however, limited studies have investigated genetic mutations in fibroadenomas [3-5].

In this study, we aimed to investigate RUNX gene family expressions in breast cancer and breast fibroadenomas.

**Material and Method**

Patient records of Mugla Sitki Kocman University Hospital Department of Pathology were searched between years 2012 and 2014. All consecutive patients who had breast cancer (31 patients), fibroadenoma (30 patients), or normal breast tissue (25 patients) were included in the study. Patient tumor stages according to the TNM classification, p53 activity, estrogen (ER), progesterone (PR) and c-erb receptor status were recorded from the patient records.

*Genetic Analysis:*

Paraffin blocks were collected from the pathology archive and tissue samples were obtained with 10- micron thickness slices. Total RNA from each sample was isolated with genomic RNA extraction sample (QIAGEN Sample & Assay Technologies, Hilden, Germany) after the tissue deparaffinization procedure. Gene expressions of RUNX1, RUNX2, and RUNX3 were measured with real time-PCR (RT-PCR).

*Statistical Analysis:*

The breast cancer patients were classified according to the tumor stage, p53 activity, and c-erb status. Since there were less number of patients in the stage I group, to increase the power of the statistical analysis, stage I and II patients (16 patients)

were tested together with stage III and IV patients (15 patients) during the analysis.

RUNX 1, 2, and 3 gene expressions between the breast cancer and fibroadenoma patients were compared with normal breast tissues by using RT<sup>2</sup> Profiler Data Analysis Software-Qiagen. The gene expression scores between the subgroups of breast cancer patients and the control group were compared with SPSS® for Windows computing program, Version 20.0.

**Results**

RUNX1 and RUNX2 gene expressions were significantly upregulated in the fibroadenoma group when compared to the normal breast tissues. Although the RUNX3 gene expression was also upregulated, it was not statistically significant (Table 1).

RUNX1, RUNX2, and RUNX3 gene expressions were upregulated in the breast cancer group, however, only RUNX2 gene expression was significantly upregulated (Table-1).

When the data was further analyzed according to the tumor stages, RUNX2 and 3 were significantly upregulated in stage I and II; but no significant upregulation was detected in stage III and IV patients (Table 2).

Regarding p53 status, RUNX1 in the p53 positive and RUNX2 in the p53 negative group were significantly upregulated. With respect to c-erb status, RUNX2 in the c-erb positive, RUNX1 and RUNX2 in the c-erb negative patients were significantly upregulated. As for the ER and PR status, RUNX2 in the ER-positive and RUNX1, RUNX2 and RUNX3 in PR negative were significantly upregulated (Table 2).

**Discussion**

When virologists were investigating mouse cell differentiation, they found that embryonal carcinoma cells could not be infected by viruses like polyomavirus before cells started to differentiate. During the differentiation phase, virologists discovered that polyomavirus enhancer binding protein 2 (PEBP2) and core binding protein 2 (CBP2) were responsible for the infection. Moreover, it was found that cells expressed PEBP2 during cell differentiation and PEBP2 was a developmental regulator. PEBP2 and CBP2 are produced via RUNX gene expressions. RUNX1, RUNX2, and RUNX3 are Runt-connected transcription factors and have essential roles in cell growth and differentiation. RUNX1 is associated with human acute leukemia, RUNX2 abates growth and activates osteoblasts, and RUNX3 is related to gastric cancer. Generally, these genes have essential roles during development of organs, and mutations occurred in these genes are the main etiologic factors of cancer development [2, 6, 7].

Table 1. RUNX1, RUNX2, RUNX3 results and p-value of breast cancer, fibroadenoma and control groups

	RUNX1		RUNX2		RUNX3	
	Cancer Group	FA Group	Cancer Group	FA Group	Cancer Group	FA Group
Avg. Delta (Ct) (2 <sup>Δ</sup> )	0.724876	2.549121	0.018195	0.018069	0.110956	0.006763
Fold change (%95 CI)	5.6718*	19.9456*	30.3764*	30.1658*	240.5953*	14.6638*
Control group	0.127804	0.127804	0.000599	0.000599	0.000461	0.000461
P-value	0.1609	0.001351**	0.032108**	0.044635**	0.124487	0.236984

\*: upregulation; \*\*: significant, p-value (comparing to control group)

FA = Fibroadenoma, Avg. = Average, Ct = Cycle threshold, CI = Confidence interval

Table 2. Detailed results of RUNX1, RUNX2, RUNX3 tests in each subgroup of breast cancer group.

Subgroups	RUNX1 Avg.Delta (Ct) (2 <sup>Δ</sup> )	Fold Change Value	p	RUNX2 Avg.Delta (Ct) (2 <sup>Δ</sup> )	Fold Change Value	p	RUNX3 Avg.Delta (Ct) (2 <sup>Δ</sup> )	Fold Change Value	p
Control group	0.127804			0.000333			0.000371		
Stage1, 2	1.396033	10.9233*	0.104	0.0166	49.8159*	0.003 <sup>μ</sup>	0.493572	1329.3758*	0.018 <sup>μ</sup>
Stage3, 4	0.786967	6.1576*	0.206	0.006014	18.0472*	0.287	0.044022	118.5683*	0.212
P53 +	0.701527	5.4891*	0.004 <sup>μ</sup>	0.017948	29.9649*	0.042 <sup>μ</sup>	0.061214	132.7345*	0.036 <sup>μ</sup>
P53 -	0.736534	5.763*	0.085	0.018199	30.3832*	0.030 <sup>μ</sup>	0.179098	388.3527*	0.152
Cerb +	0.992793	7.7681*	0.183	0.021012	35.0804*	0.027 <sup>μ</sup>	0.107774	233.6956*	0.098
Cerb -	0.586869	4.592*	0.027 <sup>μ</sup>	0.019251	32.1403*	0.012 <sup>μ</sup>	0.239631	519.6113*	0.057
ER +	0.791743	6.195*	0.147	0.019929	33.2719*	0.026 <sup>μ</sup>	0.114721	248.7593*	0.115
PR +	0.770525	6.029*	0.109	0.017327	28.9267*	0.040 <sup>μ</sup>	0.100769	218.5062*	0.135
PR -	0.594089	4.6485*	<0.001 <sup>μ</sup>	0.020457	34.1532*	0.023 <sup>μ</sup>	0.142967	310.0055*	0.033 <sup>μ</sup>

\*: upregulation; <sup>μ</sup>: significant, p value (compared to control group)  
Avg. = Average, Ct = Cycle threshold

RUNX1 has a critical role in hematopoiesis. Functional impairment of this gene causes acute myelogenous leukemia, myelodysplastic syndrome, or chronic myelogenous leukemia [8]. On the other hand, aberrations of RUNX2 is related to impaired bone development, and aberrations of RUNX3 gene is related to gastrointestinal disorders such as destruction of chief cells in the stomach or even gastric cancer. Moreover, RUNX3 plays a tumor suppressor role in the lung and it normally regulates bronchioalveolar cell differentiation [9-12]. The mechanism of destruction of tumor suppressor genes is usually ascribed to hypermethylation of the genes. Environmental factors like aging, smoking, alcohol consumption, decreased folate intake, or oxidative stress has been shown to lead hypermethylation process of the tumor suppressor genes like RUNX3 [13].

However, there is less number of studies in the literature that investigated the relationship between the RUNX gene expressions and cancer. Some of the genetic alterations in fibroadenoma were the following: P53 gene mutations, microsatellite alterations, chromosomal deletion, and Her-2/neu amplification [14-17]. Furthermore, some authors studied genetic alterations in patients with multiple fibroadenomas and found that single nucleotide polymorphism in prolactin receptor genes or an FHIT gene mutation [18-20]. To the best of our knowledge, our study is the first study that evaluates RUNX1, RUNX2, and RUNX3 gene expressions in both benign and malignant breast disease. RUNX2 gene was significantly upregulated in patients with both breast cancer and fibroadenoma in our study. In contrast, however, upregulated RUNX1 and RUNX3 gene upregulations in the breast cancer and fibroadenoma patients were not statistically significant.

Our data was further analyzed to find any relation between the RUNX gene expressions and p53, c-erb, ER, and PR status. Since RUNX1 was upregulated in p53 positive patients and RUNX2 was upregulated in p53 negative patients, we thought that RUNX1 may be a good and RUNX2 may be a bad prognostic factor. However, we could not make a conclusion because the c-erb, ER, and PR status did not support this idea. Moreover, the upregulation of RUNX2 and RUNX3 in stage I and II patients suggested that these genes may be associated with early-stage breast cancer. Furthermore, RUNX2 was also upregulated in the fibroadenoma group. These findings suggested

us that RUNX2 may be a good prognostic factor in our study, in contrast to its role in osteosarcoma or bone metastasis in breast and prostate cancer.

As a conclusion, RUNX gene family has essential roles in the cell-differentiation and has been investigated to detect their roles in cancer development. RUNX2 gene was significantly upregulated in patients with both breast cancer and fibroadenoma in our study. Further studies may clarify the relation between breast cancer and RUNX gene expressions and new approaches may be found in the treatment of patients.

**Scientific Responsibility Statement**

*The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.*

**Animal and human rights statement**

*All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.*

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**Conflict of interest**

*None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.*

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