Published in final edited form as:

Eur J Cancer. 2009 September ; 45(13): 2239–2248. doi:10.1016/j.ejca.2009.06.021.

Positive Association between Nuclear Runx2 and Estrogen-Progesterone Receptor Gene Expression Characterizes a Biological Subtype of Breast Cancer

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Abstract

Purpose—The runt-related transcription factor, *Runx2* may have an oncogenic role in mediating metastatic events in breast cancer, but whether *Runx2* has a role in the early phases of breast cancer development is not clear. We examined the expression of *Runx2* and its relationship with estrogen receptor (*ER*) and progesterone receptor (*PR*) in breast cancer cell lines and tissues.

Methods—Two human breast cancer cell lines, MCF-7 and MDA-MB-231 were transiently transfected with vectors expressing either *Runx2* or *ER* and the levels of both proteins and mRNA were examined by Western blot analysis and quantitative real-time PCR respectively. *Runx2* expression was also examined in tissue microarray sections of 123 breast cancer patients by immunohistochemistry and results were correlated with clinico-pathological characteristics.

Results—Expression of *Runx2* and *ER* was reciprocal in the breast cell culture models and *Runx2* suppressed *ERβ* but *ERα* not mRNA levels. In contrast, functional expression of *Runx2* was evident in the nucleus in 28% of the breast cancer tissues and in both early and late stages of tumor growth. Importantly, *Runx2* expression was significantly more frequent in Grade 2 compared to Grade 1 and Grade 3 tumors (48% vs 39% vs 13%) and the expression was significantly associated with *ER* ($p=0.005$), *PR* ($p=0.008$) expression in Grade 2 & 3 tumors than the Grade 1 tumors.

Conclusion—We propose that *Runx2*, *ER* and *PR* triple positivity in Grade 2 & 3 defines a biological subtype in breast cancer.

Keywords

Runx2; breast cancer; estrogen receptor; progesterone receptor; *CerbB2*

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INTRODUCTION

Breast cancer occurs due to the action of the two main female steroid hormones, estrogen and progesterone and their cognate nuclear receptors (respectively, *ER* and *PR*) that are known to be potent mitogenic factors. The pathological roles of both hormone receptors in the etiology of breast cancer have been demonstrated by *in vitro* and *in vivo* studies¹. Both *ER* and *PR* have been implicated in the initiation or promotion of breast cancer by stimulating breast cell proliferation while bypassing cell cycle checkpoints that normally prevent damage DNA in response to genotoxic insult and permitting the incorporation of random genetic mutations².

The estrogen receptor (*ER*) exists in two isoforms, *ERα* and *ERβ* that are encoded by two distinct genes, *ESR1* and *ESR2*, located in chromosomal loci at 6q25 and 14q23, respectively³. The ratio of *ERα* and *ERβ* varies between tissues and results in different physiological responses to estrogen. Breast cancer shows mostly higher levels of *ERα* and its expression is associated with more differentiated tumors and a more favorable prognosis, although the potential role of *ERβ* as a favorable prognostic indicator has also been suggested⁴. Progesterone binds to the progesterone receptor and also has two isoforms, *PR-A* and *PR-B* that differ in protein sequence and function but are encoded by the same gene. *PR-A* lacks the N-terminal 164 amino acids of *PR-B* and has only two of the three activation function domains that are present in *PR-B*. Thus, *PR-B* shows a higher transcriptional activity and acts as a more potent transcriptional activator of target genes than *PR-A*^{5, 6}. Imbalance in the ratio of these two forms of *PR* may be associated with the development and progression of breast cancer⁶⁻⁸.

The majority of breast cancers are either positive for *ER* alone or positive for both *ER* and *PR*. Patients that are both *ER* and *PR* positive have a good prognosis⁹ and are most likely to benefit from endocrine therapy¹⁰. Other forms of breast cancer such as *ER* negative/*PR* negative or triple negative breast cancers that lack *ER*, *PR* and human epidermal growth factor receptor 2 (*HER-2*) also occur, though less frequently. *HER-2* is encoded by the *CerbB2* gene (abbreviation for 'v-erb-b2 erythroblastic leukemia viral oncogene homolog 2') and is also known as the neuro/glioblastoma (c-neu) proto-oncogene that is located on chromosome 17 and encodes a 185kDa transmembrane tyrosine kinase receptor. Mutations in the *CerbB2/HER-2* gene predict poor outcome in breast cancer and the encoded protein is over expressed in at least 30% of breast cancers diagnosed¹¹.

Steroid hormones including estrogen action can enhance bone formation by blocking bone resorption by osteoclasts and/or promoting the bone anabolic functions of osteoblasts. Breast cancer that metastasizes to bone is predominantly osteolytic¹²⁻¹⁶. The Runt-related transcription factor *Runx2*¹⁷ is known to mediate the expression of proteins that support breast cancer metastasis¹⁸⁻²². *Runx2* is essential for osteogenesis and differentiation of osteoblasts²³⁻²⁴. Oncogenic properties of *Runx2* have also been demonstrated in T-cell lymphomas²⁵ and prostate cancer²⁶. Apart from oncogenic functions upon ectopic expression, endogenous expression of *Runx2* in osteoblasts is linked to control of cell growth²⁷⁻³⁰ and its expression in mammary epithelial cells may support normal breast development³¹⁻³². Other reports have suggested links between steroids and *Runx2*^{25, 33, 34}. Steroids such as glucocorticoids alter the expression of genes associated with osteoblast function at low doses³⁵, while high doses of this adrenal hormone cause bone loss and fractures^{36, 37}. Glucocorticoids have inhibitory effects on *Runx2* function by depleting its nuclear accumulation³⁸. *Runx2* has also been linked with *ERα* and both were shown to interact through the *ERα* DNA binding domain. Estrogen enhances *Runx2* activity in dose and estrogen receptor-dependent ways. Notably, deletion of the DNA binding domain of *ER*, eliminates the stimulatory effects of estrogen on *Runx2* activity³⁹. *ER* and *PR* serve as the basis for many therapeutic interventions in breast cancer and *Runx2* has been implicated as a regulator of early metastatic events in this cancer. However,

links between steroid hormone signaling and pathological functions of *Runx2* in breast tumorigenesis remain to be explored.

In this study, we investigate the relationship between *Runx2* and the two main female hormone receptors, *ER* and *PR*, as well as the oncogene *CerbB2* (*HER-2/c-neu*), in a panel of breast cancer clinical samples. Our results show that nuclear presence of *Runx2* is significantly and positively correlated with *ER-PR* positive breast cancer tissues in Grade 2 & 3 tumors. Our results provide direct clinical evidence indicating a biological role of *Runx2* in the pathology of steroid hormone related breast cancers.

MATERIALS AND METHODS

Cell Culture and Transfections

Two breast cancer cell lines, MDA-MB-231 and MCF-7 cells and an osteosarcoma cell line, Saos2 (ATCC, Manassas, VA, USA) were cultured in DMEM media supplemented with 10% fetal bovine serum. MDA-MB-231 and MCF-7 cells were transfected either with *ER* or *Runx2* pcDNA3 expression vector using Eugene 6 (Roche, Basel, Switzerland) and Lipofectamine 2000 transfection reagent (Invitrogen Corporation, CA, USA) respectively. The empty vector (pcDNA3) was used as a control in both cases. Three days post transfection, protein and RNA were extracted for Western blot analysis and quantitative real-time PCR respectively.

Western Blotting

All the three cell lines were first checked for the endogenous levels of *ER*, *PR* and *Runx2*. Osteosarcoma cells were used as *Runx2* positive controls. Cells were lysed with lysis buffer (2% SDS, 10% glycerol, 5% 2-mercaptoethanol, 0.002% bromophenol blue and 62.6mM Tris HCl, pH 6.8), briefly sonicated and pre-cleared with high speed centrifugation. Supernatants were loaded and proteins separated by 10% SDS-PAGE and transferred to a nitrocellulose membrane. Proteins were probed with anti-*Runx2* (MBL International, Woburn, MA, USA), anti-*ER*, anti-*PR* and anti- β -actin (Santa Cruz Biotechnology Inc., CA, USA) primary antibodies followed by appropriate horse radish peroxidase conjugated to anti-rabbit or anti-mouse secondary antibodies and detected with luminol on exposure to X-ray film.

Quantitative PCR

For quantitative PCR measurements, total RNA from the transfected cells (MCF-7 and MDA-MB-231) was extracted and reverse transcribed using High capacity cDNA reverse transcription kit (Applied Biosystems, USA). The cDNA (15ng) from these cells was amplified in an ABI 7300 Prism system using primers of target genes and Fermentas Maxima SYBR Green qPCR Master Mix. Cycling conditions were 10 mins at 95°C followed by 40 cycles of 15 sec at 94°C, 30 sec at 60°C and 20 sec at 72°C. GAPDH was used as an internal control. The sequences of the forward and reverse primers of the genes were as follows: GAPDH_Fwd: 5' GAGTCCACTGGCGTCTTCA 3' GAPDH_Rev : 5' GTTCACACCCATGACGAACA 3' *ER α* _Fwd : 5' CCTATCTCAGGGAGGGAAGG 3' *ER α* _Rev : 5' TCTCCAAGTCCCACTCTGCT 3' *ER β* _Fwd : 5' CACCAACGAGTGCGAGATCA 3' *ER β* _Rev : 5' TCCCCACTTTGAGGCATTTC 3' *PR*_Fwd : 5' GATTCAGAAGCCAGCCAGAG 3' *PR*_Rev : 5' GACCTTACAGCTCCCACAGG 3' *Runx2*_Fwd: 5' GGAGTGGACGAGGCAAGAGTTT 3' *Runx2*_Rev: 5' AGCTTCTGTCTGTGCCTTCTGG 3'

Patient samples

Tissue microarray (TMA) sections from 123 breast cancer patients (invasive ductal carcinomas representing all grade and stage) and 2 normal breast tissues (controls) were obtained from the department of pathology, National University Hospital, Singapore upon ethics approval (B06/006) by the institutional review board. These were constructed according to previously reported methods⁴⁰, representing all grades and stages of the commonest breast cancer (the invasive ductal carcinoma type). The median age of patients was 52 years (range 29-86). The distribution of patients according to the three most common ethnic groups in Singapore showed that they were of Chinese (81%), Malay (15%) or Indian (4%) descent. Histopathological staging of BC was based on the TNM staging system⁴¹ and grading⁴² of tumors. Patients that were alive and being followed up in the hospital after initial diagnosis and surgery and at the time of our data analyses were 91/123 (74%), those that did not follow up were 14/123 (11%) and those that died of the disease were 18/123 (15%).

Immunohistochemistry

Immunohistochemistry was performed on breast cancer TMAs constructed and stained according to our protocol described previously with minor modifications⁴⁰. Briefly, sections were microwaved at 98 °C for 20 mins in citrate buffer (pH6.0) (DAKO, Denmark) followed by peroxidase blocking for 1 hr at room temperature. Anti-*Runx2* mouse mAb⁴³ at 1: 500 dilution, *ER1D5* antibody at 1:1000, for *ER* detection, NCL-PGR1A6 at 1:1000 for *PR* and Hercep Test K5204 antibody at 1:500 for *CerbB2*, (DAKO, Denmark)⁴⁴ were used as primary antibodies, incubated for 2hrs at room temperature followed by diaminobenzidine (DAKO, Denmark) staining and detection.

Statistical analysis

Runx2, *ER*, *PR* and *CerbB2* immunostained TMA sections were visually scored by two independent observers (AG and MST) that were blinded to clinical outcome of the patients. The staining was semi-quantitatively assessed on a scale ranging from 0 to 3 (0-no staining; 1- weak staining, 2- moderate staining and 3- strong staining) following our previously published protocols for *Runx* family of proteins⁴³ and also *ER*, *PR* and *CerbB2* proteins⁴⁴. The status *CerbB2* positive and *CerbB2* negative in this study is scored by immunohistochemistry following standard practice, with +0 and +1 representing negative staining and +2 and +3 representing positive. In our hands, this scoring method in TMA is equivalent to the scoring in full sections (see reference⁴⁴) and also to HER2 status by FISH.

For statistical analyses negative and weak nuclear or cytoplasmic staining were grouped together and termed as 'negative expression' of the proteins and moderate and strong nuclear or cytoplasmic staining was termed as 'positive expression' of *Runx2*. Fisher's exact test was used to test for the association between *Runx2* expression and the clinico-pathological parameters of breast cancer. Survival analysis was performed according to Kaplan and Meier method. All statistical analyses were performed using SPSS version 16.0 for Windows (SPSS Inc., Chicago IL). The p values were from two-sided tests and p< 0.05 was considered significant.

RESULTS

Runx2 is inversely correlated to *ER* status in breast cancer cell lines

We analyzed the relationship between *Runx2*, *ER* and *PR* genes *in vitro* by selecting two breast cancer cell lines, MCF-7 and the highly metastatic MDA-MB-231. The cell lines were examined for endogenous levels of *Runx2* and *ER* and it was observed that MCF-7 was *ER* positive and negative for *Runx2* while MDA-MB-231 was *ER* negative and positive for

Runx2 expression level (Figure 1A). This finding is consistent with a requirement for *ER* in early stages of breast cancer^{2, 4} and the metastatic potential of *Runx2* in later stages^{14,18,19}.

To assess whether the relationship between *ER* and *Runx2* reflects regulatory coupling, the cells were then transiently transfected with vectors expressing either *Runx2* or *ER* and the levels of both proteins and mRNA were examined by Western blot analysis and quantitative real-time PCR respectively. In the *Runx2* negative MCF-7 cell line, transfection of *Runx2* suppressed expression of *ER* (Figure 1B). In contrast, exogenous expression of *ER* in negative MDA-MB-231 cells appreciably altered the levels of *Runx2* protein (Figure 1C). The protein data for *Runx2* and *ER* were confirmed by examination of transcripts level. The endogenous *Runx2* mRNA level was low or diminished in *ER* positive cell lines (MCF-7). In contrast, the *ER*-negative cell lines (MDA-MB-231) showed high levels of *Runx2* mRNA (Figure 2A). Interestingly, exogenous expression of *Runx2* in MCF-7 cells exhibited low levels of *ERα*, *ERβ* and *PR* (Figure 2B) indicating that *Runx2* effects the expression of the two *ER* isoforms. Similarly, when MDA-MB-231 cells were over expressed with *ERα*, the *Runx2* mRNA level was suppressed (Figure 2C). Thus, *ER* and *Runx2* expression is inversely correlated in these cell lines, consistent with their respective roles in early and late stages of breast cancer, and *Runx2* is capable of attenuating *ER* levels.

***Runx2* is localized in the nucleus and cytoplasm of breast tissues**

To begin understanding the clinical relationships between steroid hormone responsive breast tumors and *Runx2*, we performed *Runx2* immunostaining and analyzed the localization of the protein in a spectrum of breast tumor clinical samples. Of the 123 tumors, *Runx2* nuclear expression was detected in 14 (11%) cases (Figure 3a), cytoplasmic expression in 24 (19%) cases (Figure 3b) and concurrent nuclear and cytoplasmic expression was observed in 20 (16%) cases (Figure 3c). There was no expression of *Runx2* in 65 (53%) cases. Two normal tissues that were considered as controls showed weak nuclear expression only, consistent with previous data suggesting a role for *Runx2* in specific stages of normal breast epithelial development³¹.

Based on the known role of *Runx2* as a nuclear transcription factor, we propose that nuclear expression of *Runx2* reflects a functionally active form of the protein, whereas its cytoplasmic localization indicates loss of function in gene regulation at least in a basal state. We do not exclude the possibilities that cytoplasmic *Runx2* may provide a reservoir of sequestered *Runx2* that might translocate to the nucleus only upon stimulation, or simply represents an inactive bystander molecule. We interpreted concurrent expression of *Runx2* in both nucleus and cytoplasm as indicating that the protein has retained its transcriptional function, and thus is at least in part active in gene regulation.

***Runx2* nuclear protein is strongly associated with *ER* —*PR* expression in Grade 2 & 3 breast cancer**

Table 1 shows the correlation of *Runx2* nuclear expression with the pathological parameters in the breast tumors. There was no significant correlation between *ER*, *PR* and *Runx2* nuclear expression in the breast tumors although *Runx2* nuclear protein was more evident in *CerbB2* negative tumors than the *CerbB2* positive tumors (70% vs 30%) that was nearly statistically significant (p=0.092),..

The *ER* and *PR* status in the tumors, when compared with pathological grade showed that *ER* was positive for (95%) grade 1 tumors (G1), positivity was 81% in grade 2 (G2) tumors and 47% in grade 3 (G3) tumors that was statistically significant (p<0.0005) (Figure 4a). Similar relation was also observed between *PR* and tumor grades (91% vs 76% vs 37%; p<0.0005) (Figure 4b). This inverse relation is consistent with previous studies and consistent

with loss of estrogen-responsiveness in late stage breast tumors (44). Expression of *CerbB2* (HER-2/c-neu) is elevated in tumors of higher grades (15% vs 40% vs 68%; $p < 0.0005$).

We next examined the expression of *Runx2* in different grades of breast cancer. The nuclear expression of *Runx2* varied significantly across the three grades of breast cancer with the highest expression in G2 tumors (Figure 5). Of the 31 tumors that showed *Runx2* nuclear expression, 48% were G2, 13% were G3 and the remaining 39% represented G1 cancer (Table1). The *Runx2* expression was then correlated with *ER* and *PR* positivity in different tumor grade and a statistically significant association was observed between them in G2 & G3 tumors compared with G1 tumours (Table 2). However, *CerbB2* showed a negative correlation with *Runx2* expression in G2 & G3 tumors which was also significant (Table2). In other words, *Runx2* was more frequently expressed in *CerbB2* negative tumors than the positive ones (39% vs 17%). There were no significant differences between *Runx2* expression and different breast cancer stages or with axillary lymphnode metastasis. *Runx2* cytoplasmic expression also did not show any significant difference when compared with the pathological parameters. Our statistical analyses did not show any correlation with patient survival. This is fully consistent with the predominant intermediate grade of *Runx2* expression in breast cancer.

DISCUSSION

There are breast cancer conditions as reflected by cell lines in our study that are *ER* positive and *Runx2* negative as observed in MCF-7 and those that are *ER* negative and *Runx2* positive in MDA-MB-231 cell lines. Reciprocal relationship between *Runx2* and *ER* expression was observed at both the mRNA and protein level suggesting that high levels of *Runx2* may be effecting the expression of *ER* in MDA-MB-231 cell lines. Because *Runx2* and *ER* can synergize in biological processes (e.g., they are both bone anabolic), one key question is whether there is an *in vivo* transition in breast cancer in which both factors can co-exist. We tested this by co-staining breast cancer clinical samples for both *ER* and *Runx2* and assessed the biological role of *Runx2* in all grades of this disease together with the hormone receptors.

Runx2 and *ER* expression results were similar to those of the cell lines in G1 tumors and G3 tumors while in G2 tumors, *Runx2*, *ER* and *PR* expression were increased significantly and there was a significant positive association between *Runx2* nuclear protein and *ER* and *PR* expression. These data suggest that *Runx2* is expressed early on during tumor growth and development.

A link between estrogen and *Runx2* has been reported through an increase in the number of *Runx2* expressing cells⁴⁵. Selective *ER* modulators, raloxifene having different modes of action from estrogen enhanced *Runx2* promoter activity⁴⁶. To bind to the target gene promoters, nuclear import of transcription factors is an essential element of the molecular pathways and when these proteins are present in the cytoplasm, they are functionally inactive and may act as bystanders or as a reservoir of unstimulated forms. Runx activity is regulated by tissue—specific expression and also those elements that control its subcellular localization. Cytoplasmic and nuclear localization has been shown for Runx3 in gastric cancer⁴⁷ and colon cancer⁴⁸ and *Runx2* in osteosarcoma cells⁴⁹. Besides nuclear and cytoplasmic expression of *Runx2*, our study also showed concurrent expression of this protein in the two subcellular components in 16% of the tumors and more frequently in the G2 tumors. This reflects ectopic induction of *Runx2* expression during breast cancer progression. Although *Runx2* expression was prevalent during tumorigenesis of mammary epithelial cells as evidenced by our breast cancer tissues, only a subset of tumors showed nuclear localization that would enable it to regulate its target genes.

Strikingly, the nuclear staining correlates with “ER positivity” suggesting that both factors synergize to stimulate tumor growth and development at one or more specific stages. Our observation that 32 % of ER+ breast cancer clinical samples express *Runx2* (n=88) is consistent with a patho-physiological role of ectopic *Runx2* expression. However, because only 28% of breast cancer tissue exhibits nuclear localization of *Runx2* (n=123), it appears *Runx2* is not actively participating in gene regulatory events, although we cannot exclude the possibility that *Runx2* may perturb gene regulatory events by its cytoplasmic localization (e.g., sequestering of key co-factors like Smad and Yap that transduces TGF-beta and Src/Yes related signals respectively).

ER and *Runx2* are both known to contribute to carcinogenesis independently. However, both proteins can also synergize at the molecular level in a manner that may reinforce their biological functions in the pathology of breast cancer. The co-expression of *ER* and *Runx2* that we have established in this study suggests that both proteins may have biological synergism that may be further supported by molecular synergy. *In vitro* studies have shown that the expressions of *ER* versus *Runx2* are inversely correlated⁵⁰, parallel to what we have observed. But the novelty of our study is that we also found a subset of breast cancer that showed co-existence of *ER* and *Runx2* suggesting the transition from positive to negative or *vice versa* as a gradual process.

Our results show that the *in vivo* disease is much more heterogeneous than the perceptions based on cell culture studies, highlighting the importance of analyzing clinical samples to understand the pathological linkages between different classes of transcription factors like *ER* and *Runx2*. Indeed, our full analysis showed cases with this reciprocal relation but, in addition, there were three important subclasses of breast cancer: *ER+/PR+/Runx2-*, *ER+/PR+/Runx2+* and *ER-/PR-/Runx2+* (in order of disease progression). We propose that *ER+/PR+/Runx2+*, triple positivity defines a biological subtype in breast cancer.

In summary, our results stress the idea that solid tumors including breast cancer, are not biologically homogenous disease and defining the biological nature of potential subgroups allows a better understanding of the disease. Finally, it is interesting to note that a defining biomarker of metastasis such as *Runx2* is predominantly expressed in G2 breast cancers. Further investigation of its relationship with *ER* & *PR* may lead to a better understanding of the molecular processes related to breast cancer metastasis.

ACKNOWLEDGEMENTS

We would like to thank Dr Doris Mayer, German Cancer Research Center, Germany for the kind gift of ER expression plasmid. Our study was supported by SCS grants MN05 and MN077 (to MST), NIH grants P01CA82834 (to G.S.S.) and AR049069 (to AJvW.) and Lee Kuan Yew Postdoctoral Fellowship awarded to DTL.

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Figure 1.

(A) Endogenous protein expression levels of ER and *Runx2* in breast cancer cell lines, MCF-7 and MDA-MB-231 [A less aggressive cell line, MCF-7 expressed ER but much lower levels of *Runx2*. Conversely, a highly metastatic, MDA-MB-231 was ER negative but showed an elevated level of *Runx2* expression. Saos2 was included as a positive control for *Runx2* expression. β -actin protein was used as a loading control]. (B) Transfection of *Runx2* in the *Runx2* negative MCF-7 cell line, suppressed expression of ER. (C) Exogenous expression of ER in ER negative MDA-MB-231 cells, downregulated *Runx2* expression level.

Figure 2.

(A) Endogenous levels of *Runx2* mRNA in breast cancer cell lines, MCF-7, MDA-MB-231 [Osteosarcoma cell line, Saos 2 was used as a positive control] (B) Quantitative RT-PCR graph functional consequences of *Runx2* up and down regulation in MCF-7 cells [*Runx2* over expression in MCF-7 cells showed a similar trend at the transcript level as observed in the protein levels in figure 1b] (C) Quantitative RT-PCR graph showing the *Runx2* mRNA level in MDA-MB-231 cells. [Exogenous over expression of ER α in MDA-MB-231 cells down regulate transcript levels of *Runx2*]

Figure 3.
Runx2 immunostaining in breast cancer tissues showing (A) nuclear staining (B) cytoplasmic staining and (C) concurrent nuclear and cytoplasmic staining

Figure 4. ER and *PR* immunostaining in G2 breast cancer tissues. (A) ER positive staining and (B) *PR* positive staining

Figure 5.
Runx2 positive expression in G2 breast cancer tissue.

TABLE 1Relationship of *Runx2* nuclear expression and pathologic features of breast cancer

Pathologic features of breast cancer	Total Cases N=123	Runx2 positive	Runx2 negative	p value
<i>Tumor size</i>		N=33	N=90	0.532
<=2cm	46	15	31	
>2-5cm	69	16	53	
>5cm	8	2	6	
<i>Axillary</i>		N= 33	N= 86	0.521
<i>Metastasis</i>				
positive	45	14	31	
negative	74	19	55	
indeterminate	4			
<i>ER</i>		N=34	N=89	0.101
positive	88	28	60	
negative	35	6	29	
<i>PR</i>		N=34	N=89	0.188
positive	83	26	57	
negative	40	8	32	
<i>CerbB2</i>		N=30	N=75	0.092
positive	45	9	36	
negative	60	21	39	
indeterminate	18			
<i>Histologic</i>		N=31	N=81	0.005*
<i>Grade</i>				
G1	42	12	30	
G2	33	15	18	
G3	37	4	33	
NA	11			
<i>Stage</i>		N=34	N=88	0.758
I	36	11	25	
II	60	15	45	
III	19	5	14	
IV	7	3	4	
indeterminate	1			

TABLE 2Correlation between ER, *PR* and *Runx2* nuclear expression in breast tumor grades

Tumor Grade Type	<i>Runx2</i> positive (%)	<i>Runx2</i> negative (%)	p value
G1	N=12	N=30	
<i>ER</i>			
positive	27	73	0.492
negative	50	50	
G2&G3	N=19	N=51	
<i>ER</i>			
positive	39	61	0.005*
negative	8	92	
G1	N=12	N=30	
<i>PR</i>			
positive	26	74	0.319
negative	50	50	
G2&G3	N=19	N=51	
<i>PR</i>			
positive	39	61	0.008*
negative	10	90	
G1			
<i>CerbB2</i>	N=8	N=19	
positive	25	75	0.826
negative	30	70	
G2&G3			
<i>CerbB2</i>	N=19	N=49	
positive	17	83	0.041*
negative	39	61	

*
p value <0.05 is statistically significant