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Molecular constitution of breast but not other reproductive tissues is rich in growth promoting molecules: A possible link to highest incidence of tumor growths

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ABSTRACT

In the current study we tested if highest incidence of benign as well as cancer growths in breast tissue is due to constitutive molecular composition of this tissue. To delineate the molecular basis, we compared the expression of nine functional gene modules (total 578 genes) that regulate major positive growth and negative inhibitory signals in normal breast with two other reproductive tissues, ovary and uterus. We present data to demonstrate that breast tissues constitutively have very highly elevated levels of several growth promoting molecules and diminished levels of inhibitory molecules which may, in part, contribute for highest incidence of tumor growths in this tissue.

1. Introduction

Numerous surveys have established that the incidence of benign as well as malignant growths is highest in breast among all male and female reproductive and non-reproductive tissues. Surveys show that in the USA alone, about 1–1.5 million benign and 180 000–200 000 cancer cases of the breast are reported per year and breast cancers account to a third of all male and female cancers combined [1–3]. Based on experimental, clinical and epidemiological studies it is widely believed that estrogen promotes benign as well as malignant growths in breast tissues. Although estrogens are known to mediate a wide variety of complex seemingly unlinked biological processes in a diverse range of reproductive and non-reproductive tissues, it is not known why mitogenic actions of estrogens are implicated as a cause of developing benign as well as malignant growths only in breast but not other target tissues. Interestingly, the incidence of benign and malignant growths in ovary and uterus, the primary sites of estrogen production and action, respectively, are substantially lower than breast. Although estrogens are also implicated in promoting the ovarian and uterine cancers, anti-estrogen treatments have little benefit to these patients.

It is largely not known why tumor growths are highest in breast and why estrogen promotes the genesis and progression of cancers only in breast but not other target tissues. To delineate the molecular bases for the above, we compared the expression of nine functional gene modules (a total of 578 genes), which regulate major positive growth and negative inhibitory signals, in normal breast with normal ovary and uterus tissues. They were: (1) growth factors, growth factor binding proteins and growth factor receptors ($n = 177$), (2) steroid receptor co-activators ($n = 20$), (3) steroid receptor co-repressors ($n = 10$), (4) kinases ($n = 54$), (5) tumor suppressors ($n = 30$), (6) oncogenes ($n = 124$), (7) apoptosis regulators ($n = 71$), (8) genes that regulate estrogen metabolism ($n = 88$), and (9) hormone receptors ($n = 4$). We selected the above functional gene modules because altered expressions of these are known to directly or indirectly support or suppress tumor growths.

We present results here to demonstrate for the first time that breast tissue constitutively has highly elevated levels of several molecules that promote cell proliferation, survival and angiogenesis which are either absent or at far diminished levels in other

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reproductive tissues. Breast also has constitutively diminished levels of negative signaling molecules that inhibit proliferation and promote apoptosis, differentiation, and tumor suppression in comparison with other reproductive tissues. The molecular constitution of breast tissue may, in part, contribute for highest incidence of tumor growths in this tissue.

2. Materials and methods

ABC staining system and primary antibodies against EGFR, EGFL6, ERα, and LTBP1 were from Santa Cruz Biotechnologies. Anti-NCOA4 antibodies were from Abnova Corp. Taiwan. Labeled Envision+® polymer-HRP and DAB were from Dakocytomation. The Taqman® Gene Expression Assay reagents were obtained from Applied Biosystems. The ID# for EGFL6, EGFR, TUSC3, NCOA4, FGF-1, LTBP1 and GHR were Hs00170955_m1, Hs00193306_m1, Hs00 394808_m1, Hs00428328_m1, Hs00265254_m1, Hs00386448_m1 and Hs00174872_m1, respectively.

2.1. Tissue samples

Normal breast tissues were obtained from women undergoing reduction mammoplasty for cosmetic reasons with no prior history of breast cancer. Normal ovary and uterus tissues were also obtained from women undergoing surgery for non-cancerous conditions and the absence of any abnormal cells in the collected tissues was verified by histological staining. All the tissues were collected from Howard University Hospital Surgical Pathology, immediately after surgery and stored at −80 °C until use. Formalin fixed paraffin embedded normal breast (reduction mammoplasty), ovary and uterus tissues were obtained from Howard University pathology tissue archives. The samples were from both premenopausal and post-menopausal women.

2.2. Micro-array analyses and real-time Q PCR

RNA extraction, purification and cDNA preparations were performed as previously described [4,5]. Gene expression analysis by micro-arrays was performed on U133A Human GeneChip (Affymetrix) that represents 22 283 biological oligonucleotides including normalization controls as previously described [6]. A total of three runs were performed for each of normal ovary, breast and uterus tissue samples. Each sample for micro-array analysis was prepared by pooling equal amounts of four different individual tissue RNA samples. Quantitative real-time PCRs were performed in ABI Prism GeneAmp 7900HT Sequence Detection System at a modified 50% Ramp rate as previously described [6–8] using gene specific assays-on-demand reagents from Applied Bio-Systems. All the samples were amplified in triplicate and real-time PCRs were repeated four times and normalized to the copy numbers of the housekeeping gene, GAPDH. Absolute quantifications of GAPDH, wild type ERα, wild type ERβ, various isoforms of ERα and ERβ mRNA copy numbers by RT real-time Q PCR were performed as previously described [7,8].

2.3. Immunohistochemical staining

Expression of selected number of genes was studied at protein levels by Immunohistochemistry (IHC) using the previously established procedures [5,6,9,10]. Briefly, the slides were deparaffinized, antigens retrieved in 10mM citrate buffer by heating for 25 min in a steamer and endogenous peroxides blocked by incubating with 3% H_2O_2 in methanol for 20 min as previously described [9,10]. The slides were blocked with blocking solution and incubated with primary antibodies for 90 min. The blocking buffer for LTBP1, EGFL6, and EGFR was 1% BSA, 0.1% Fish Gelatin, 0.1% Triton X-100 and 0.05% Tween 20 in 1 × PBS and for NCOA4 and ERα the above solution without fish gelatin. The primary antibody dilutions for LTBP1, EGFL6, ERα, EGFR and NCOA4 were 1:200, 1:200, 1:100, 1:50, and 1:100, respectively. The EGFL6 and LTBP1 antibodies were diluted in 1.5% donkey serum in PBS. The EGFR, ERα and NCOA4 antibodies were diluted in PBS. The secondary antibodies for EGFL6 and LTBP1 were goat–ABC staining system. For EGFR it was anti-rabbit labeled Envision+® polymer-HRP. For NCOA4 and ERα they were anti-mouse labeled Envision+® polymer-HRP. The peroxidase substrate for all was DAB (DakoCytomation, #K3468). All the stained slides were scored independently by two pathologists for staining intensity and scored using an arbitrary scale of 0–3, 3 being the slide with the highest staining intensity and score 0 for slides with no stain.

2.4. Statistical analyses

The expression of 9 functional gene modules (a total of 578 genes, Supplementary Table 1) in breast tissue was compared with ovary and uterus tissues. In order to identify genes that were differentially expressed in breast tissue in comparison with ovary and uterus tissue, logarithmic ratios (to the base 2) of breast/ovary and uterus were calculated by comparison analysis using GCOS for all 18 combinations of breast and ovary and uterus pairs. Mean of all ratios, one sample *t*-test *P*-value, number of Increase, and Decrease calls were calculated for each gene. Differentially expressed genes in breast tissue were selected in comparison with ovary and uterus tissues based on: (1) the qualities of the 18 ratios, (2) the *t*-test *P*-values from the 18 ratios, (3) the mean of all 18 signal ratios and (4) the numbers of Increase and Decrease calls. The four conditions were set as in the following. To control the quality of the ratios, at least 10 out of the 18 ratios must satisfy one of the three conditions: a "change" *P*-value <0.003 (Increase) or >0.997 (Decrease) or a signal detection *P*-value <0.065. The *t*-test *P*-value was less than 0.001. The mean of all 18 signal ratios (change fold) was at least 1.5 and the numbers of Increase and Decrease calls were not the same. Statistical analyses of RT real-time Q PCR and IHC data were performed using Wilcoxon test and one way ANOVA. The results were considered significant if the *P*-values were <0.05.

3. Results

To delineate the molecular bases for highest incidence of tumor growths in breast tissue, we first performed global gene expression of normal female human breast tissue and two other normal reproductive tissues, ovary and uterus, the primary sites of estrogen synthesis and action, respectively. We next compared the expression levels of nine functional gene modules which regulate major positive growth and negative inhibitory signals, in breast with ovary and uterus. They were: (1) growth factors, growth factor binding proteins and growth factor receptors ($n = 177$), (2) steroid receptor co-activators ($n = 20$), (3) steroid receptor co-repressors ($n = 10$), (4) kinases ($n = 54$), (5) tumor suppressors ($n = 30$), (6) oncogenes ($n = 124$), (7) apoptosis regulators ($n = 71$), (8) genes that regulate estrogen metabolism ($n = 88$), and (9) hormone receptors ($n = 4$) (Supplementary Table 1).

3.1. Breast tissue had similar expression levels of over 90% of genes compared with ovary and uterus

At the statistical significance level specified in methods, of the 578 genes compared all but 53 showed surprisingly similar expression levels in all three tissues. Of the 53 differentially expressed genes, 25 were over-expressed and 28 were under-

expressed in breast in comparison with ovary and uterus (Supplementary Table 2). The functional categories of the differentially expressed genes are given in Table 1.

3.2. Breast tissue was constitutively rich with a number of growth promoting positive signaling molecules – genes that inhibit apoptosis and promote cell proliferation, survival, and angiogenesis

Three growth factors, EGF, EGFL6 and IGF-1, which require estrogen for their actions and stimulate cell cycle progression by activating ras-raf-MAPK1-erk cascade, activating PI3K/AKT survival pathways and various transcription factors such as c-fos, c-jun, c-myc, STATS and inactivating apoptosis inducers, are abundantly expressed in breast tissues. EGFR that regulates multiple biological processes such as proliferation, angiogenesis, inhibition of apoptosis, and promote tumor cell motility, adhesion, invasion and metastasis was expressed in large amounts only in breast tissue in addition to abundant amounts of its ligands. EGFR protein was totally absent in ovary and uterus tissues (Fig. 2A, lower panel). MET oncogene that has receptor tyrosine kinase activity and other functional properties similar to EGFR was also at elevated levels in breast tissue. To complement higher expression levels of EGFL6, IGF, EGF, EGFR and MET, higher levels of signal transmitter adaptor family members, CRK and VAV3, were at elevated levels in breast. Breast tissues also constitutively had abundant levels of growth factors, VEGF-B, VEGF-D, ECGF-1, FGF-1 and FGF-2, some of which correlate to estrogen levels [11] and are known to promote angiogenesis by stimulating endothelial cell proliferation, adhesion, migration, invasion and morphogenesis. The expressions of the above growth factors were either absent or at markedly diminished levels in ovary and uterus.

To complement the signals transmitted by the above growth factors, breast tissues also had higher expression levels of kinases required for nucleic acid synthesis (UMP-CMPK), assembly of components of the transcription machinery (HIPK2) and translation initiation (MKNK2). In addition, expression of cell survival promoter (SGKL) and apoptosis inhibitor (CFLAR) were also at elevated levels in this tissue. Unexpectedly, GHR that is required for breast development and functionally linked to IGF production was the most highly over-expressed among the differentially expressed genes in breast. GHR was at markedly at far diminished levels in ovary and uterus (Fig. 1 and Supplementary Table 2).

3.3. Breast tissue was constitutively very poor in negative signaling molecules – genes that promote differentiation and apoptosis and suppress proliferation

Two IGF binding proteins, IGFBP4 and IGFBP5, which limit the availability of IGF and independently inhibit DNA synthesis, arrest cell cycle progression and promote differentiation, were poorly expressed in breast tissue. Three TGFβ binding proteins, LTBP1, LTBP3 and LTBP4, known to be required for the storage, activation and targeting of TGFβ to its cell surface receptor were under-expressed in breast. Under-expression of one of the above proteins, LTBP1, in breast tissue at the mRNA levels could be seen in Fig. 1 and at protein levels in representative tissues by IHC in Fig. 2B (lower panel). Diminished levels of the above binding proteins could limit the availability of proliferation inhibiting and differentiation and apoptosis promoting TGFβ levels in breast tissue. Other growth factors expressed at lower levels in breast were involved in either promoting differentiation (FGF-9, EBAF, PSIPI, CTGF, PDGFC, PDGFRA) or coordinating intercellular communications (CTGF). The JUN-D proto-oncogene, which was known to reduce cell proliferation, inhibit angiogenesis and promote differentiation, was lowest in breast. Two other differentiation promoting proto-oncogenes, MAFF-1 and FOSB-1 were also at lower levels. Four tumor suppressors, GLTSCR2, TUSC3, WT1, and TSC22, were expressed at significantly diminished levels in breast. In addition to lower levels of differentiation promoting molecules, breast tissue had under-expression of genes involved in inducing apoptosis (CASP9), nuclear transport of proteins necessary for apoptosis (CSEIL), and transcription of genes required for apoptosis response (DPF2).

3.4. Breast tissue was not significantly different from ovary and uterus in the levels of genes that regulate estrogen metabolism or signal transmitting molecules

Interestingly, we did not observe any differences in the expressions of 88 genes involved in estrogen metabolism or signaling in breast. The level of ERα, the major player in mediating estrogen signaling, was not significantly different under the statistical criteria applied. However, higher relative levels of several ER isoforms (Supplementary Fig. 1A) were observed in breast tissues. Neither ERβ nor progesterone receptor were differentially expressed. Interestingly ERα protein staining was observed in about 10% of ductal epithelial cells of the breast and predominantly in the nucleus where it is active in contrast to predominantly inactive cytosolic staining in ovary and uterus cells (Supplementary Fig. 1B). Of the several steroid receptor co-activators and co-repressors, only NCOA4 that increases the activity of ERα and other steroid/nuclear receptors was at highly elevated levels in the breast tissue (Fig. 2B, upper panel). The staining pattern of NCOA4 complemented ERα in that it is present predominantly in nucleus where it is active in breast cells in contrast to predominantly cytosolic staining where it is inactive in ovary and uterus cells. Other than ERα and NCOA4 nuclear localization, breast tissue did not appear to be significantly different in the levels of other molecules involved in estrogen synthesis, metabolism or classical estrogen signaling pathways.

Table 1
Functional categories of genes significantly over-, and under-expressed in breast in comparison with ovary and uterus.

#	Functional category	Genes significantly over-expressed	Genes significantly under-expressed
1.	Growth factor mediated signaling (growth factors, growth factor binding proteins and growth factor receptors)	EGF, EGFL6, EGFR, FGF-1(acidic), FGF-2, IGF-1, VEGF-B, FIGF (VEGF-D), ECGF-1, and TGFBR3	LTBP1, LTBP3, LTBP4, IGFBP4, IGFBP5, PDGFC, HDGFRP3, FGF9, FGF-2R, EBAF (TGFbeta4), PDGFRA, PSIP1 and CTGF
2.	Oncogenes	VAV3, RAB7L1, CRK, RAB5C, and MET	MAFF, RAB31, JUN-D, RAB23, JUN and FOSB
3.	Tumor suppressors	FAT	GLTSCR2, TUSC3, WT1 and TSC22
4.	Steroid receptor co-activators	NCOA4	None
5.	Steroid receptor co-repressors	None	None
6.	Kinases	MKNK2, SGKL, PGK1, HIPK2, UMP-CMPK	AKAP13, MLP
7.	Apoptosis	CFLAR, CASP1	CASP9, DPF2, CSE1L,
8.	Hormone receptors	GHR	None
9.	Genes involved in estrogen metabolism	None	None

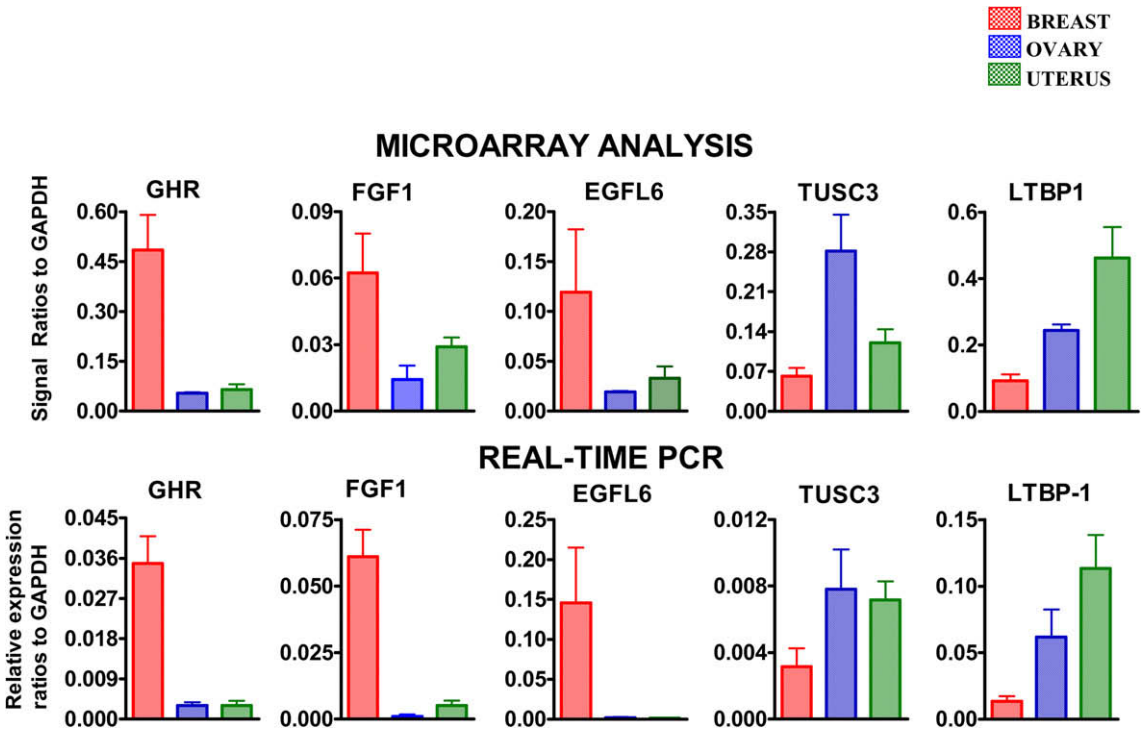

Fig. 1. The expression of GHR, FGF-1, EGFL6, TUSC3 and LTBP1 in breast is distinct from other reproductive tissues. To corroborate the micro-array data, the expressions of three over-expressed genes (GHR, FGF-1 and EGFL6) and two under-expressed genes (LTBP1 and TUSC3) were determined by RT Q real-time PCR using Taqman® gene specific assays-on-demand reagents from Applied Biosystems in individual cDNAs from 12 normal ovary, 22 normal uterus and 20 reduction mammoplasty tissues as described in Section 2. The signal values of the above five genes normalized to GAPDH by micro-array analyses and RT Q real-time PCR are shown schematically. Heights of the boxes and bars represent mean and standard deviations, respectively.

3.5. Elevated expression of EGFR, EGFL6, GHR, FGF-1, NCOA4 and diminished expression of TUSC3 and LTBP1 in breast tissue by micro-array analyses could be independently verified by Q real-time PCR and or immunohistochemistry (IHC)

To verify the micro-array data, three over-expressed genes, GHR, FGF-1, EGFL6 and two under-expressed genes, TUSC3 and LTBP1, were examined in a total of 22 normal uterus, 20 normal breast (reduction mammoplasty) and 12 normal ovary tissues by RT real-time QPCR using gene specific assays-on-demand reagents from Applied Bio-systems and normalized to the copy numbers of the house keeping gene, GAPDH. Analyses of the Q real-time PCR data by Wilcoxon test confirmed that the expression of the above genes was significantly different in breast in comparison with ovary and uterus (*P*-values for the above markers were 0.00, 5.4e−011, 2.4e−6, 0.0054 and 7e−6, respectively) and in concordance with micro-array data (shown schematically in Fig. 1).

The micro-array data were further validated by IHC for three over-expressed genes, EGFL6, EGFR, and NCOA4 and one under-expressed gene, LTBP1 (Table 1), in formalin fixed paraffin embedded archival normal ovary ($n = 30$), normal uterus ($n = 27$) and reduction mammoplasty tissues ($n = 29$) using their specific antibodies. The staining grades of each of the above markers in breast were compared with ovary and uterus by Wilcoxon test. We found the above marker expressions at protein levels were consistently different in breast in comparison with ovary and uterus (*P*-values 5.9e−7, 1e−8, 1.2e−5 and 2.2e−14, respectively, for the above markers) as observed by micro-array analyses establishing an excellent concordance between mRNA and protein levels. IHC staining of EGFL6 and EGFR in representative tissues is shown in Fig. 2A and NCOA4 and LTBP1 in Fig. 2B. Although EGFR mRNA was detected in ovary and uterus (Supplementary Tables 1 and 2), we could detect its protein only in breast but none in ovary and uterus (Fig. 2A, lower panel). We also detected EGFL6 protein only in breast (Fig. 2A, upper panel) as observed at mRNA levels (Fig. 1 and Supplementary Tables 1 and 2). We observed heavy NCOA4 protein staining in both nucleus and cytosol of breast cells, but significantly lower in ovary and uterus cells (Fig. 2B, upper panel). LTBP1 protein was abundantly expressed in ovary and uterus but was significantly lower in breast (Fig. 2B, lower panel) confirming micro-array data. We also evaluated an association between menopausal status and expression of the above proteins in all the three tissues by ANOVA and did not find any correlation.

4. Discussion

In the current study we have addressed a fundamental question of why of all human tissues breast is so highly prone to developing tumor growths and of all target reproductive tissues, estrogen is known to drive the tumor growths only in this tissue. We have tested whether the molecular constitution of breast but not other reproductive tissues is conducive to foster estrogen dependent tumor growths. For our study, we selected ovary and uterus tissues for comparative purpose because these tissues are the primary sites of estrogen synthesis and action, respectively. In addition, the incidences of malignant growths in the above tissues are far (about 10- and 5-fold, respectively) lower than breast.

We studied the expression of nine functional gene modules that regulate major positive growth and negative inhibitory signals and could directly or indirectly drive or suppress tumor growths. The results presented here show that breast tissue is surprisingly not very different from ovary and uterus in the levels of molecules that regulate estrogen metabolism or signaling. The only molecule which showed significant difference was NCOA4 that could increase the transcription of genes under steroid hormone control

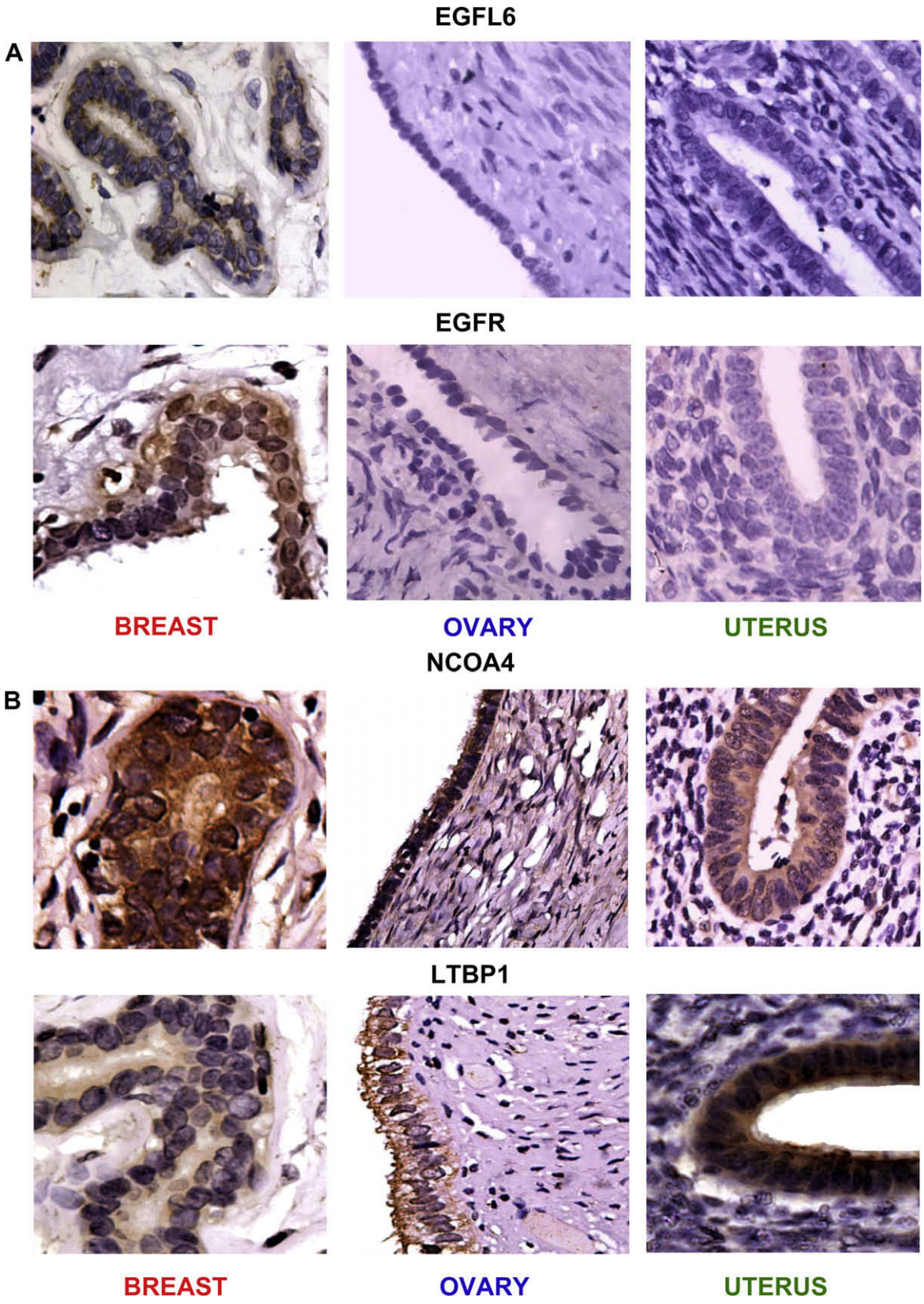

Fig. 2. EGFL6 and EGFR proteins were detected only in breast but not in ovary or uterus and NCOA4 and LTBP1 proteins were differentially expressed. The micro-array data were further validated by immunohistochemistry for three over-expressed genes, EGFL6, EGFR, and NCOA4 and one under-expressed gene, LTBP1, in formalin fixed paraffin embedded archival normal ovary ($n = 30$), normal uterus ($n = 27$) and reduction mammoplasty tissues ($n = 29$) using their specific antibodies as described in Section 2. Representative tissues for the above markers are shown. EGFR and EGFL6 proteins were totally absent in ovary and uterus (2A). NCOA4 and LTBP1 were differentially expressed (2B).

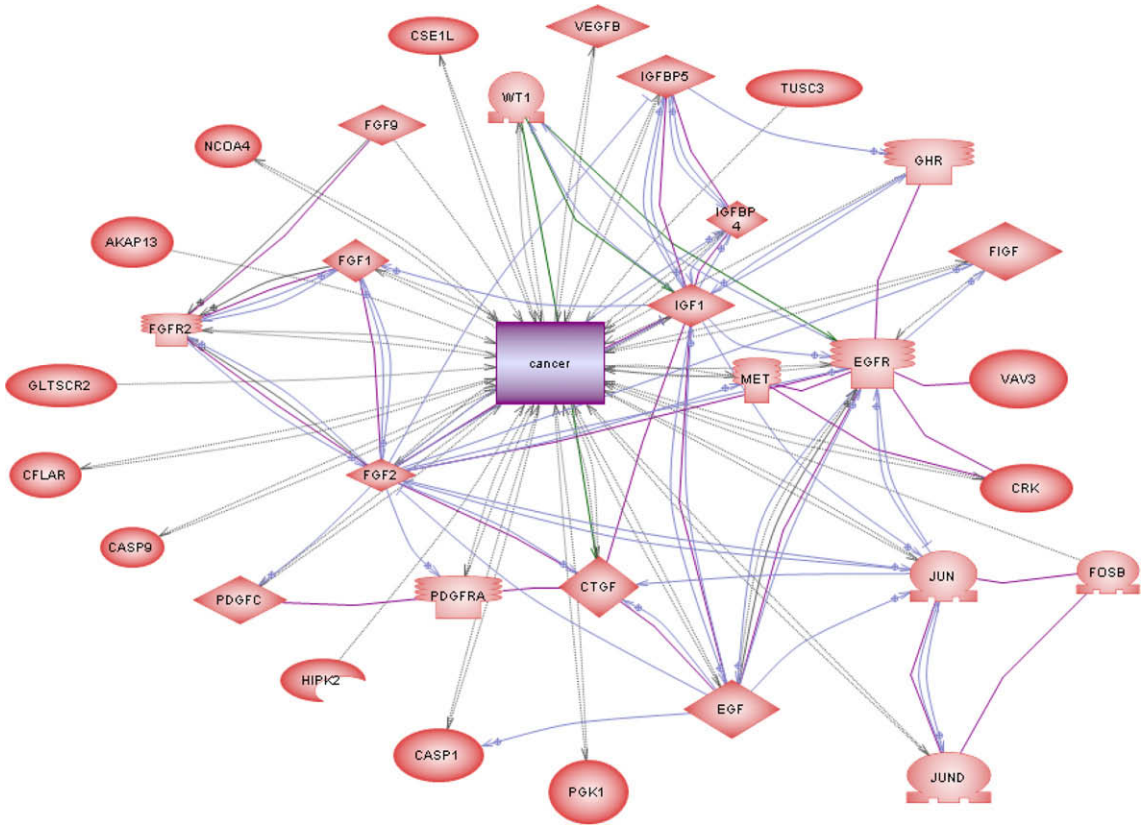

Fig. 3. Molecular pathway analysis using the Pathway Studio 6.2 program. The differentially expressed gene list (53 entities) (Supplementary Table 2) was imported into the Pathway Studio program. Using the pathways/groups enriched analysis method, the above Cancer Pathways involving 33 entities was on the top of the list with *P*-value of 2.41×10^{-31} are shown.

by interacting with estrogen receptor and several other steroid receptors. Interestingly, the staining of NCOA4 was predominantly in nucleus in the breast cells where it is active in contrast to predominantly cytosolic staining where it is inactive in ovary and uterus cells (Fig. 2B, upper panel). ERα staining pattern complemented NCOA4 in breast tissue in that it is predominantly in the nucleus where as in ovary and uterus it was mostly in inactive form in the cytosol (Supplementary Fig. 1B). The nuclear localization of these two molecules could significantly contribute in increasing the transcription of genes under estrogen control and tumorigenesis in breast but not in ovary and uterus.

Although we did not observe significant differences with respect to the molecules that directly influence estrogen action, growth factor signaling molecules through which estrogen could indirectly increase growth and tumorigenesis are at far exceeding levels in breast but are low or totally absent in other reproductive tissues. Three major growth factors, IGF-1, EGF and EGFL6, that require estrogen for activation and increase cell proliferation through several mechanisms and contribute to tumorigenesis were at far exceeding levels in breast but were absent or at diminished levels in other tissues (Supplementary Tables 1 and 2). In addition, two tyrosine kinases, EGFR and MET, known tumor promoters were also constitutively at elevated levels in breast. Unexpectedly, GHR that increases IGF production and involved in breast development was expressed in large amounts in breast tissue but absent in ovary and uterus (Fig. 1). Two IGF binding proteins, IGFBP4 and IGFBP5, which limit the availability of proliferation promoting IGF-1, were at far diminished levels in breast. Three TGFβ binding proteins, LTBP1, LTBP3, and LTBP5, which limit the availability of differentiation promoting, tumor suppressing TGFβ were also at far diminished levels in breast cells. Our results also show that angiogenesis promoting growth factors, VEGF-B, VEGF-D, ECGF-1, FGF-1 and FGF-2, were abundantly expressed in breast compared to other tissues. To complement the growth factor mediated signaling, breast tissue was also rich with molecules that function in signal transmission (CRK, VAV3), nucleic acid synthesis (UMP-CMPK), transcription (HIPK2) and translation (MKNK2). The above observations demonstrated that breast tissue is very rich with growth factors or other related molecules that increase cell proliferation through several mechanisms and contribute to tumorigenesis. Other reproductive tissues have diminished levels of these molecules. Our observations also suggested that growth factor signaling could play a major role in predisposing the breast tissue for tumor growths. Analyses of the micro-array data on all the differentially expressed genes in breast tissue using Pathway Studio program provide support for the role of growth factors in tumor growths in this tissue. Pathways generated showed intricate connections between the differentially expressed genes (shown in Fig. 3) and growth factors that increase cell proliferation through several inter-connected mechanisms could, in part, contribute to susceptibility of breast tissue to tumorigenesis. Estrogen carcinogenesis in breast tissue could be predominantly through growth factors. Other reproductive tissues could be less prone to tumor growths because of diminished growth factor signaling.

The results presented here also show that in addition to rich proliferation promoting environment, breast tissues have poorly expressed negative inhibitory/tumor suppressing molecules. They include differentiation promoting, proliferation inhibiting growth factors (FGF-9, EBAF, PSIPI, CTGF, PDGFC and PDGFRA) and proto-oncogenes (JUN-D, MAFF-1 and FOSB-1). Other poorly expressed negative signaling molecules include tumor suppressors (LTSCR2, TUSC3, WT1, and TSC22) and apoptosis promoters (CASP9, CSEIL,

DPF2). While we have screened for major signaling molecules here, it is possible that there may be other molecules that could contribute to tumorigenesis in breast.

In summary, our results show for the first time that breast molecular constitution is significantly different from other reproductive tissues with respect to the balance in positive growth and negative inhibitory signaling molecules. Overall, breast tissues have abundant expressions of genes that promote cell proliferation, survival, and angiogenesis and significantly diminished levels of negative signaling molecules that inhibit proliferation and promote apoptosis, differentiation, and tumor suppression in comparison with other reproductive tissues. Breast tissue molecular constitution may, in part, contribute in supporting tumor growths and other reproductive tissues are less vulnerable because of low expression of growth factors and other molecules through which estrogen could promote tumorigenesis. The molecular information presented above will form a foundation for understanding differential effects of endogenous as well as exogenous factors on different tissues.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2009.08.021.

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