

Short Communication

Sam-Pointed Domain Containing Ets Transcription Factor in Luminal Breast Cancer Pathogenesis

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Abstract

We previously described frequent overexpression of Sam-pointed domain containing Ets transcription factor (SPDEF), also known as PDEF, in human breast cancer, and suggested a role for this transcription factor in breast tumor progression. To seek evidence in support of this hypothesis, the MCF-12A breast epithelial cell line was transfected with an SPDEF expression plasmid or with control vector plasmid and the transfected cells tested for their tumorigenic growth *in vivo*. The data showed that SPDEF expression in MCF-12A cells induced accelerated tumor growth in severe combined immune deficient mice compared with vector-transfected MCF-12A cells. Furthermore, Gene Expression Omnibus and Oncomine databases were mined to determine any correlation between SPDEF expression levels and clinical

outcome. High SPDEF expression correlated with poor overall survival of patients with estrogen receptor+ breast cancer, in three independent data sets. In contrast, little correlation was observed between SPDEF expression and cancer relapse or remote metastases. SPDEF expression was further found to be restricted to tumors arising in the luminal epithelial lineage including estrogen receptor+ luminal subtype breast tumors, Her2/neu-positive tumors, and apocrine carcinomas. In contrast, little SPDEF expression was found in the basal subtype of breast tumors. Based on these results, we hypothesize that SPDEF has a function in the specification of the progenitor cells of the luminal epithelial lineage that become targets of oncogenesis in luminal breast cancer. (Cancer Epidemiol Biomarkers Prev 2009;18(6):1899–903)

Introduction

Recent work on the cellular hierarchy in normal mammary gland development shows that the three mature cell types including the basal myoepithelial, luminal ductal, and luminal alveolar cells arise from regulated specification, proliferation, and differentiation of progenitor and stem cell precursors (1, 2). In parallel with this understanding, there has been an equally remarkable evolution in our understanding of the origin of the heterogeneity of human breast cancer, i.e., the basal versus luminal breast cancer subtypes seem to be derived from the corresponding normal cells with distinct gene expression profiles (3, 4). Despite these advances, molecular mechanisms that regulate lineage specification, proliferation, and differentiation in the normal breast and that underlie breast cancer heterogeneity remain poorly understood.

Changes in the activities of specific transcription factors are known to specify cell fates along alternate lineages in the hematopoietic developmental programs (5). For example, GATA-1 and PU.1 transcription factors control differentiation of the common precursors along erythroid versus myeloid lineage, and GATA-3 and T-bet transcription factors specify restricted differentiation to TH2 versus TH1 lineage, respectively (reviewed in ref. 5). Moreover, aberrant activation and expression of these and other transcription factors is a significant contributor to the development of leukemia in man (5). These observations highlight the fine balance in transcription factor regulation that separates normal from neoplastic development and emphasize the importance of studying the role of specific transcription factors in normal developmental processes and in cancer. Despite such importance, our understanding of the role of specific transcription factors in normal mammary gland development remains rudimentary. Moreover, the dynamics of activation and/or inactivation of specific transcription factors in the genesis of luminal versus basal breast cancer subtypes remains to be elucidated.

Sam-pointed domain containing Ets transcription factor (SPDEF), such as PU.1 described above, belongs to the Ets family of transcription factors that regulate many of the developmental processes including cell lineage specification, proliferation, differentiation, angiogenesis, and apoptosis, and are instrumental in the oncogenesis caused by their aberrant activation and expression

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(reviewed in refs. 6, 7). We previously reported a detailed study of SPDEF protein expression during breast tumor progression and showed that individual samples vary greatly with regard to the intensity of staining and the percentage of positively staining cells. Nevertheless, increase in the expression of SPDEF protein was frequently observed in progression from benign breast tissue to carcinoma and this was further confirmed in the large majority of matched samples of benign breast and tumor tissues from same patients (8). Such breast tumor-associated expression of SPDEF raised the potential for its role in mammary gland development and in the pathogenesis of breast cancer. Moreover, the potential of SPDEF in lineage specification and/or in luminal versus basal breast cancer development seemed intriguing. In this article, data are presented that support a role for SPDEF in the pathogenesis of luminal breast cancer.

Materials and Methods

Cell Lines and Mice. The MCF-12A human mammary epithelial cell line was obtained from American Type Culture Collection and cultured in Supplier's suggested medium in 5% CO₂ at 37°C. CB17/Icr female severe combined immunodeficient^{-/-} mice were purchased from Taconic Farms.

Generation of SPDEF-Expressing MCF-12A Cells and Testing of Tumorigenicity in Severe Combined Immunodeficient Mice. The full-length coding region of SPDEF was amplified from normal human prostate RNA by reverse transcription-PCR. The SPDEF-specific primers used in the amplification were as follows: 5'-ATG GGC AGC GCC AGC CCG GGT C-3' (forward) and 5'-TCA GAT GGG GTG CAC GAA CTG GT-3' (reverse), respectively. The PCR product was cloned into the TA cloning site of pcDNA3.1/v5-His TOPO mammalian expression vector (Invitrogen). The orientation and sequence of the cloned cDNA was confirmed by DNA sequencing. Ten micrograms of the SPDEF expression plasmid were transfected into the MCF-12A cell line by using Lipofectamine 2000 (Invitrogen) transfection reagent and by using the manufacturer's suggested procedure. Stable transfectants were obtained by selection with G418 sulfate. As control, MCF-12A cells were also transfected with the vector plasmid and stable transfectants selected similarly. SPDEF expression in transfectants was tested by Western blotting using the procedures and the anti-SPDEF antibody described previously (8). Cells (10⁶) of each of SPDEF-expressing and control vector-transfected MCF-12A transfectants were injected s.c. into groups of three severe combined immunodeficient mice each and tumor growth monitored.

Results

Transfection of SPDEF into the MCF-12A Breast Epithelial Cell Line Enhances Its Tumorigenic Growth in Immunodeficient Mice. SPDEF expression plasmid was generated by amplifying the coding region of SPDEF by reverse transcription-PCR, followed by its insertion into pcDNA3.1/v5-His TOPO mammalian expression vector (see Materials and Methods). This expression plasmid was transfected into MCF-12A cells. Also, MCF12A cells

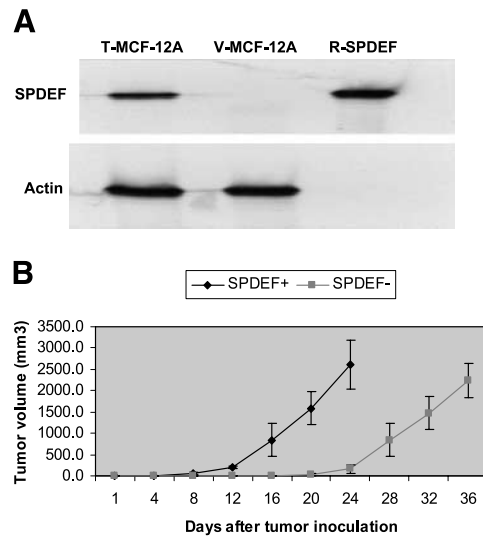


Figure 1. **A.** Testing of SPDEF expression in SPDEF expression plasmid or vector plasmid-transfected MCF-12A cells by Western blotting. *Top*, the results of screening the blot with anti-SPDEF antibody; *bottom*, the results of screening the same blot with anti-actin antibody. SPDEF-transfected (*left lane*) and vector-transfected MCF-12A cells (*middle lane*) are shown. *R-SPDEF*, the recombinant SPDEF protein. **B.** Tumorigenicity of SPDEF-expressing and SPDEF-lacking MCF-12A cells. Cells (10⁶) of each of SPDEF-expressing MCF-12A (*SPDEF+*) and control vector-transfected MCF-12A (*SPDEF-*) transfectant were injected s.c. into groups of three severe combined immunodeficient mice each and tumor growth monitored. The tumor volume at various time points is the average for three mice from each group.

were transfected with control vector plasmid and stable transfectants isolated by selection with G418. Expression of SPDEF protein in the transfected cells was tested by Western blotting, using an anti-SPDEF antibody described previously (8). As shown in Fig. 1A, a specific band at 46 kDa was found in the SPDEF plasmid-transfected cells but not in the vector-transfected cells. The tumorigenicity of SPDEF-expressing cells and SPDEF-lacking vector-transfected cells was then tested in severe combined immunodeficient mice. As shown in Fig. 1B, SPDEF-transfected MCF-12A cells showed faster growth kinetics i.e., about 12 days in advance of the vector-transfected MCF-12A cells.

Higher SPDEF Expression Correlates with Poor Overall Survival for Breast Cancer Patients with Estrogen Receptor+ Tumors. To seek further evidence for a role of SPDEF in breast cancer progression, we searched the Gene Expression Omnibus⁶ and Oncomine databases (9) that contain the gene expression profiling and clinical outcome data from several previously published studies (10-15) and looked for any correlation between SPDEF expression levels and clinical outcome. Eleven data sets had disease-specific survival information available and six of those also contained information on SPDEF expression. According

⁶ <http://www.ncbi.nlm.nih.gov/geo>

to the clinical data, the six data sets could be classified into three categories: overall survival (two data sets), relapse/metastases-free survival (three data sets), and combination of the two (one data set). Due to adequate numbers of patients with estrogen receptor (ER)+ breast tumors, only those were included in this analysis. The results show that in three independent data sets (10-12), higher SPDEF expression was associated with poor overall survival for patients (Fig. 2A-C). A similar correlation, however, was not found between higher SPDEF expression and relapse or metastases (Fig. 2D-F). The significant correlation between high SPDEF expression and poor overall survival for patients in three separate data sets, nonetheless, provides an important evidence for a role for SPDEF in breast cancer progression.

SPDEF Expression is Primarily Restricted to Breast Tumors Arising from Luminal Epithelial Lineage. To obtain additional insights into the expression characteristics of SPDEF in different subtypes of breast cancer, SPDEF expression data were extracted from the gene expression profiling data sets from Minn et al. (14) and Farmer et al. (16). As shown in Fig. 3A, SPDEF was not expressed in

any of the 16 tumors of basal subtype. In contrast, 20 of 27 (74%) of luminal tumors, 6 of 6 (100%) of apocrine tumors, and 21 of 23 (91%) Her2/neu-positive tumors expressed SPDEF. These results show restriction of SPDEF expression to tumors arising from the luminal epithelial lineage.

Discussion

The role of SPDEF in breast cancer has been a subject of controversy (17, 18). Specifically, transfection of MDA-MB231 and other malignant breast tumor cell lines including MDA-MB157, BT-549, and MDA-MB436 with SPDEF was reported to reduce their growth, motility, and invasiveness *in vitro* (17). However, in view of the data shown in Fig. 3A, a concern with transfecting these tumor cell lines with SPDEF is that they are of basal origin (19-21), wherein SPDEF is not expressed naturally and may lack a function, so that transfecting SPDEF into the basal tumor cell lines may not yield a physiologic outcome. Moreover, further increase in the malignancy of these already malignant cell lines may not be feasible, although SPDEF has a

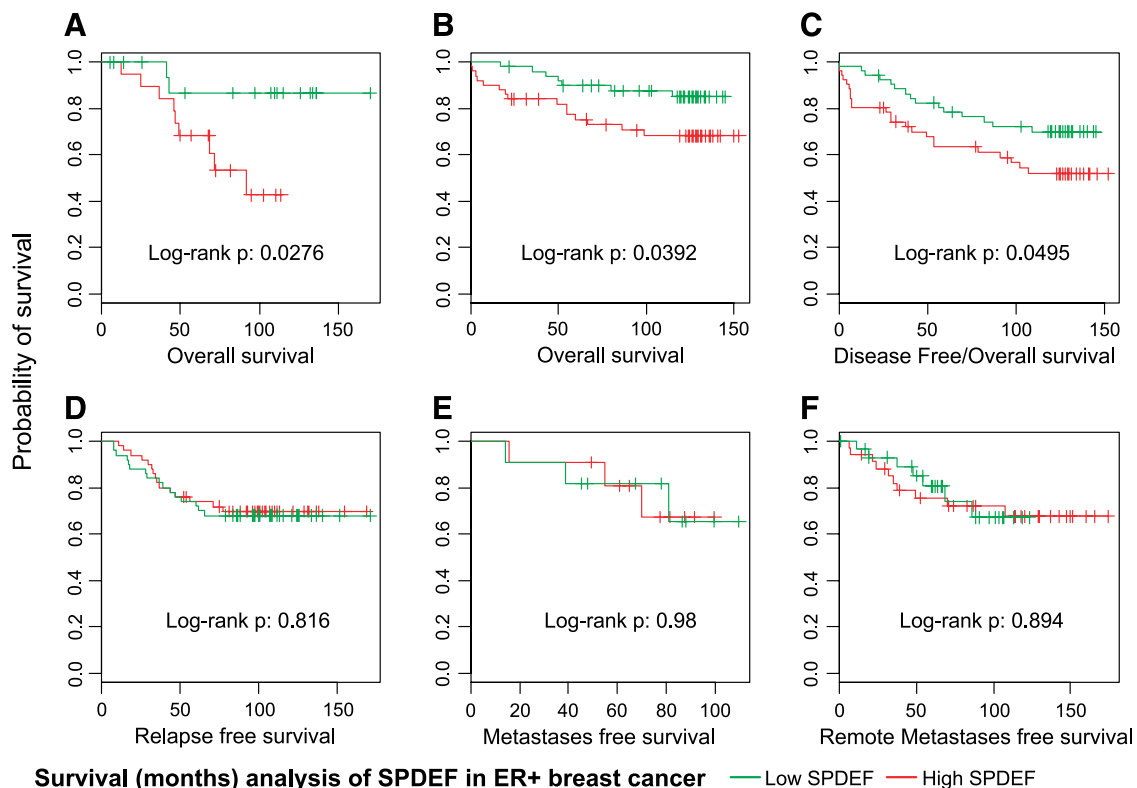


Figure 2. Survival analysis with SPDEF expression in patients with ER-positive breast cancer. Six data sets with survival data and SPDEF expression data were studied. For each data set, the low 25% quartile of SPDEF-expressing samples was categorized as low-expression samples and the high 25% quartile as high-expression samples. Comparison of these low and high expression cohorts from the data sets **A** (of Chin et al.; ref. 10) and **B** (of Miller et al.; ref. 11) showed significantly poor overall survival for the SPDEF high-expression cohort. Also, in the data set **C** of Ivshina et al. (12) that includes mixed clinical outcome data on overall survival and metastases-free survival, a significant difference was observed between low and high SPDEF expression cohorts. The log-rank test P values are also shown in the figure. In contrast, no significant difference was observed between low and high SPDEF expression cohorts with respect to relapse-free or metastases-free survival of patients in the data sets **D** (of Wang et al.; ref. 13), **E** (of Minn et al.; ref. 14), and **F** (by Sotiriou et al.; ref. 15). Cox proportional-hazard regression analysis, which treats SPDEF expression as a continuous dependent variable, was also done and similar significance levels were obtained for those six data sets.

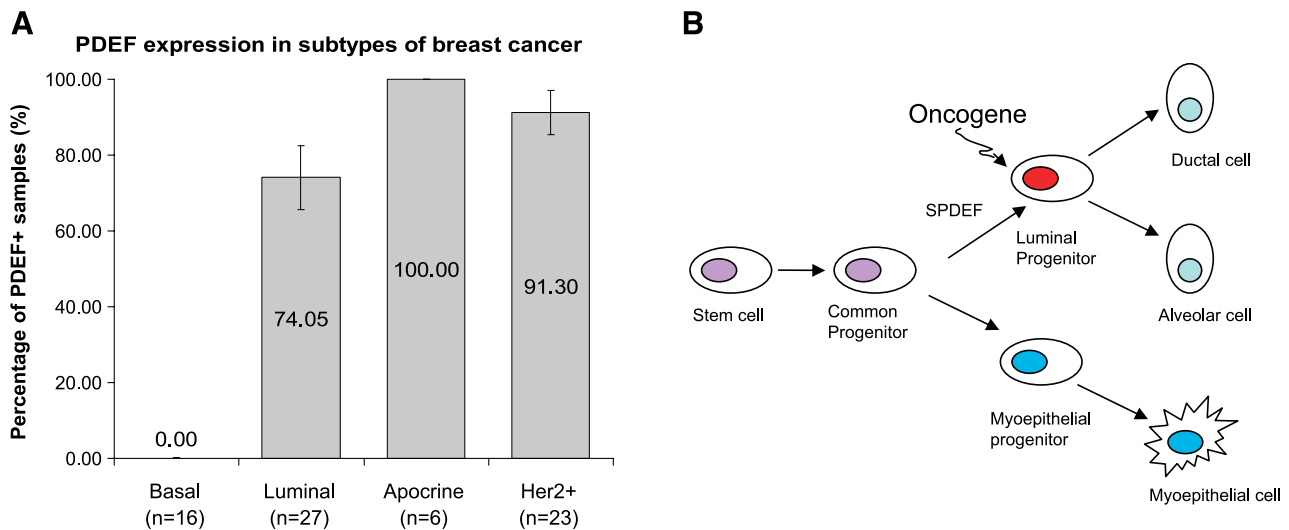


Figure 3. **A.** SPDEF expression in different subtypes of breast cancer. As shown, SPDEF was not expressed in any of the 16 tumors of basal subtype. In contrast, 20 of 27 (74%) of ER-positive/Her2-negative tumors, 6 of 6 (100%) of apocrine tumors, and 21 of 23 (91%) Her2-positive tumors expressed SPDEF. A sample was considered SPDEF-positive if a majority of the probe sets were scored “present” for SPDEF. The error for the proportion is computed with assumption of binomial distribution. **B.** A working model of SPDEF function in normal and neoplastic development in the mammary gland. SPDEF expression is depicted to occur in luminal progenitor cells that are targeted by rat neu, polyoma virus-middle T antigen, and possibly other oncogenes. Additionally, aberrant SPDEF expression may occur in hormone receptor-positive tumors to promote their de-differentiation and progression.

role in tumor progression. Similar paradoxical results were obtained when ER, another luminal lineage-associated transcription factor, was transfected into the MDA-MB231 basal tumor cell line, and behaved as an apparent tumor suppressor (22).

It seemed more appropriate to use nontransformed or low malignancy breast epithelial cell lines to discern the role of SPDEF in breast tumor progression. Indeed, when SPDEF was transfected into the nontransformed MCF-10A breast epithelial cell line in conjunction with Her2/neu or CSF/CSF-1R, it induced anchorage independent growth, motility, and invasiveness in the transfected cells *in vitro* (18). These results together with our *in vivo* data derived from the low malignancy breast epithelial cell line MCF-12A (Fig. 1B), support a role for SPDEF in breast tumor progression.

The data in Fig. 2 show a significant correlation between high SPDEF expression and poor overall survival for patients with ER+ breast tumors, in three independent data sets. These findings in conjunction with restricted expression of SPDEF to luminal breast tumors (Fig. 3A) show an important role for SPDEF in the pathogenesis of luminal breast cancer, which comprises >80% of the newly diagnosed breast tumors.

Because of their dominant role in cell fate specification and differentiation, function of individual transcription factors in mammary gland development has been the subject of recent inquiries. Specifically, work on GATA-3 transcription factor is noteworthy as it shows a crucial role for this transcription factor in luminal lineage specification and differentiation (23). Similarly, Elf-5 transcription factor was found necessary for lobuloalveolar development during pregnancy (24). Based on the lack of SPDEF expression in basal tumors (Fig. 3A), in basal tumor cell lines including MDA-MB231, MDA-MB157, BT-549, and

MDA-MB436 (17) and in nontransformed basal cell lines MCF-10A (18) and MCF-12A,⁷ SPDEF function also seems to be restricted to the luminal epithelial cell lineage and in the pathogenesis of luminal breast cancer. The data in Fig. 2 support this notion since high SPDEF expression in ER+ luminal breast tumors was found to be associated with poor overall survival of patients. Additionally, high SPDEF expression was reported in mammary tumors arising in the two transgenic mouse lines including the rat neu and the polyoma virus-middle T antigen transgenic lines (25).⁸ Mammary tumors from these mice are of luminal origin (26) and, interestingly, seem to arise from immature luminal precursors because they lack the expression of ER and FoxA1 (26), two of the molecular markers of mature luminal epithelial cells. This suggests that rat neu and polyoma virus-middle T antigen oncogenes preferentially target the SPDEF-expressing immature progenitors to produce aggressive luminal mammary tumors. Our previous observation with human breast tumors also supports this idea because frequent elevated expression of SPDEF was observed in Her2/neu-overexpressing breast tumors (8). On the basis of these observations, we propose a working model of SPDEF function in the specification of progenitor cells of the luminal epithelial lineage (Fig. 3B), which are apparently targeted by oncogenes to initiate breast cancer. Additionally, in tumors initiated through hormone receptor signaling, aberrant overexpression of SPDEF may occur and promote tumor de-differentiation and progression.

⁷ Our unpublished data.

⁸ M. Aneja and A.K. Sood, our unpublished data.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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References

- Kordon EC, Smith GH. An entire functional mammary gland may comprise the progeny from a single cell. *Development* 1998;125:1921–30.
- Stingl J, Eirew P, Ricketson I, et al. Purification and unique properties of mammary epithelial stem cells. *Nature* 2006;439:993–7.
- Sorlie T, Tibshirani R, Parker J, et al. Repeated observation of breast tumor subtypes in independent gene expression data sets. *Proc Natl Acad Sci U S A* 2003;100:8418–23.
- Weigelt B, Horlings HM, Kreike B, et al. Refinement of breast cancer classification by molecular characterization of histological special types. *J Pathol* 2008;216:141–50.
- Orkin SH. Diversification of haematopoietic stem cells to specific lineages. *Nat Rev Genet* 2000;1:57–64.
- Sharrocks AD. The ETS-domain transcription factor family. *Nat Rev Mol Cell Biol* 2001;2:827–37.
- Gutierrez-Hartmann A, Duval DL, Bradford AP. ETS transcription factors in endocrine systems. *Trends Endocrinol Metab* 2007;18:150–8.
- Sood AK, Saxena R, Groth J, et al. Expression characteristics of PDEF support a role in breast and prostate cancer progression. *Hum Pathol* 2007;38:1628–38.
- Rhodes DR, Kalyana-Sundaram S, Mahavisno V, et al. Oncomine 3.0: genes, pathways, and networks in a collection of 18,000 cancer gene expression profiles. *Neoplasia* 2007;9:166–80.
- Chin K, DeVries S, Fridlyand J, et al. Genomic and transcriptional aberrations linked to breast cancer pathophysiology. *Cancer Cell* 2006;10:529–41.
- Miller LD, Smeds J, George J, et al. An expression signature for p53 status in human breast cancer predicts mutation status, transcriptional effects, and patient survival. *Proc Natl Acad Sci U S A* 2005;102:13550–5.
- Ivshina AV, George J, Senko O, et al. Genetic reclassification of histologic grade delineates new clinical subtypes of breast cancer. *Cancer Res* 2006;66:10292–301.
- Wang Y, Klijn JG, Zhang Y, et al. Gene-expression profiles to predict distant metastasis of lymph-node-negative primary breast cancer. *Lancet* 2005;365:671–9.
- Minn AJ, Gupta GP, Siegel PM, et al. Genes that mediate breast cancer metastasis to lung. *Nature* 2005;436:518–24.
- Sotiriou C, Wirapati P, Loi S, et al. Gene expression profiling in breast cancer: understanding the molecular basis of histologic grade to improve prognosis. *J Natl Cancer Inst* 2006;98:262–72.
- Farmer P, Bonnefoi H, Becette V, et al. Identification of molecular apocrine breast tumours by microarray analysis. *Oncogene* 2005;24:4660–71.
- Turner DP, Moussa O, Sauane M, Fisher PB, Watson DK. Prostate-derived ETS factor is a mediator of metastatic potential through the inhibition of migration and invasion in breast cancer. *Cancer Res* 2007;67:1618–25.
- Gunawardane RN, Sgroi DC, Wrobel CN, Koh E, Daley GQ, Brugge JS. Novel role for PDEF in epithelial cell migration and invasion. *Cancer Res* 2005;65:11572–80.
- Charafe-Jauffret E, Ginestier C, Monville F, et al. Gene expression profiling of breast cell lines identifies potential new basal markers. *Oncogene* 2006;25:2273–84.
- Neve RM, Chin K, Fridlyand J, et al. A collection of breast cancer cell lines for the study of functionally distinct cancer subtypes. *Cancer Cell* 2006;10:515–27.
- Kenny PA, Lee GY, Myers CA, et al. The morphologies of breast cancer cell lines in three-dimensional assays correlate with their profiles of gene expression. *Mol Oncol* 2007;1:84–96.
- Levenson AS, Jordan VC. Transfection of human estrogen receptor (ER) cDNA into ER-negative mammalian cell lines. *J Steroid Biochem Mol Biol* 1994;51:229–39.
- Asselin-Labat ML, Sutherland KD, Barker H, et al. Gata-3 is an essential regulator of mammary-gland morphogenesis and luminal-cell differentiation. *Nat Cell Biol* 2007;9:201–9.
- Oakes SR, Naylor MJ, Asselin-Labat ML, et al. The Ets transcription factor Elf5 specifies mammary alveolar cell fate. *Genes Dev* 2008;22:581–6.
- Galang CK, Muller WJ, Foos G, Oshima RG, Hauser CA. Changes in the expression of many Ets family transcription factors and of potential target genes in normal mammary tissue and tumors. *J Biol Chem* 2004;279:11281–92.
- Herschkowitz JI, Simin K, Weigman VJ, et al. Identification of conserved gene expression features between murine mammary carcinoma models and human breast tumors. *Genome Biol* 2007;8:R76.