Stat5 in breast cancer: potential oncogenic activity coincides with positive prognosis for the disease

Itamar Barash*
Institute of Animal Science, ARO, The Volcani Center, Bet Dagan, Israel
*To whom correspondence should be addressed. Tel: +972 8 9484418; Fax: +972 8 9475075; Email: barashi@agri.huji.ac.il

Introduction

Nuclear localization of signal transducer and activator of transcription (Stat) 5 marks good prognosis in estrogen receptor/progesterone receptor-positive breast tumors. This positive characteristic is counteracted by studies in laboratory animals demonstrating that deregulated Stat5 activity may convert proper mammary development into a latent oncogetic process. Tumorigenesis is initiated during the parity cycles, most probably during pregnancy, when the activated Stat5 antagonizes or manipulates parity’s protective mechanisms. For example, it can alter the differentiation/proliferation balance, induce growth hormone signaling, cause specific alteration in chromatin structure, inhibit tumor-suppressor activity and induce DNA damage that counteracts the enhanced DNA-damage response exerted by parity. Palpable tumors develop after a latent period from individual cells. This happens in the estropausal period in transgenic mice maintaining deregulated Stat5 activity in the mammary gland, or during involution, months after transplantation of transplanted cells with constitutively active Stat5. Candidate vulnerable cells are those which maintain high nuclear Stat5 activity. Due to the hazardous outcome of deregulated Stat5 activity in these cells, such as induced DNA damage or high cyclin D1 activity, the gland is prone to transformation. The developing tumors are mostly adenocarcinomas or their subtypes. They are estrogen receptor-positive and maintain a specific Stat5 gene signature that allows tracking their inducer. From a clinical point of view, deregulated Stat5 activity represents a genuine risk factor for breast cancer. Monitoring Stat5 activity during vulnerable periods and developing specific tools for its suppression in breast epithelial cells could potentially limit new incidence of the disease.

Abbreviations: BLG, β-lactoglobulin; DDR, DNA-damage response; ER, estrogen receptor; GH, growth hormone; Jak, Janus kinase; PR, progesterone receptor; Stat, signal transducer and activator of transcription; STAT5, transgenic Stat5; STAT5ca, constitutively active transgenic Stat5; TAD, transactivation domain.

from maintaining mammary gland homeostasis, through induction of parity-dependent tissue transformation, to affecting tumor phenotype and characteristics. In node-negative ER/progesterone receptor (PR)-positive tumors, these are the basis for favorable prognosis. The latent cryptic breast cancer risk that is associated with deregulated Stat5 activity during specific periods in parity has not been fully recognized in humans. Consequently, specific clinical tool has been introduced to monitor Stat5 activity in order to decrease susceptibility to the disease.

Stat5 unifies two transcription factors. Stat5a and Stat5b are encoded by juxtaposed, yet distinct genes that diverged due to phylogenetic gene duplication of a common ancestor, represented by the drosophila single Stat (Stat93E) gene (10,11). The two Stat5 variants share structural similarities between themselves and with the five other members of the Stat family. Five main domains contribute to the backbone of the Stat molecules: a coiled-coil domain, a DNA-binding domain, SH3 and SH2 domains and a transactivation domain (TAD) at the C terminus. The latter shows the highest variability between the two Stat5 variants. It interacts with regulatory factors such as the histone acetyltransferases CBP and p300 (12–15), thus conferring Stat5 to epigenetic and environmental regulation. A natural truncated Stat5 variant lacking its C terminus has also been identified (16).

Post-translational modifications activate Stat5. These include glycosylation, ubiquitination, serine/threonine phosphorylation and tyrosine phosphorylation by interacting proteins. Tyrosine phosphorylation on Y694 or Y699 of Stat5a and Stat5b, respectively, is mandatory for Stat5 nuclear localization and induction of specific gene expression. Briefly, ligand binding to its cognate receptor induces receptor phosphorylation by Janus kinase (Jak) 2, which leads to recruitment of Stat5. Stat5 is phosphorylated on the C-terminally located tyrosines, dissociates from the receptor complex, dimerizes by reciprocal interaction of phosphorylated Y694/699 with the SH2 domain of a sister molecule, translocates to the nucleus and binds target sites (TTC(A>T)GGAA) in individual gene promoters. The mechanism is detailed in several excellent reviews (14,17). Interestingly, Zhu and et al. (18) recently identified a discrepancy between Stat5 binding and transcriptional regulation. Increasing Stat5 concentrations greatly increased the number of genes bound by Stat5. However, Stat5 binding to upstream promoter sequences did not automatically convey STAT5 control over those genes. In fact, only a small fraction of the genes acquired Stat5 control, suggesting that specific contexts are essential for the execution of Stat5 activity. Non-genomic functions of Stat5 have also been recently reported in the cytoplasm, aimed at preserving the structure and function of the golgi apparatus–ER unit (19).

Stat5 is ubiquitously expressed in mammalian tissues. Its activity has been mainly characterized in the hematopoietic cell system and the epithelial cells of the mammary gland/breast. Controlled Stat5 activity in the hematopoietic system is essential for proper development and survival of the lymphoid B and T cells. Its deregulation is associated with the development of Hodgkin’s lymphoma and Burkitt’s lymphoma in humans (13) and, via the kinase BCR–ABL, plays a critical role in acute lymphoblastic leukemia. Accordingly, complete deletion of Stat5ab prevented the development of leukemia in primary recipients. However, it did not affect its progression to lymphoid blast crisis or abolish established B-cell acute lymphoblastic leukemia (20,21), thus limiting Stat5 involvement to confined stages of the disease. Interestingly, in certain myeloproliferative neoplasms, such as BCR-ABL-positive CMLand KIT (D816V-positive mastocytosis), phosphorylated Stat5a is mainly localized in the cytoplasm. The mechanism that prevents nuclear accumulation of phosphorylated...
Stat5a in the BCR-ABL-positive cells involves Src family kinases that interfere with nuclear translocation of the activated Stat5a (22). It is still unclear whether the unusual distribution of activated Stat5a in the presence of Src family kinases drives tumor progression or is protective.

In the mammary gland, Stat5 expression and particularly its activity are synchronized with the reproductive cycle, a phenomenon that is more salient with Stat5a. During puberty, basal activity of Stat5 maintains secondary ductal and side branching (23). Starting at mid pregnancy, Stat5 activity is induced by elevating levels of the lactogenic hormone prolactin toward an early lactation peak and then declines upon involution. Via numerous interactions with growth factors and cytokines, Stat5 induces and maintains epithelial cell proliferation, differentiation and milk-protein gene expression (24) and reviewed in [11,14,17]). Stat5 activity is also essential for epithelial cell survival. By controlling epithelial cell number during lactation and involution, it adapt s overall milk production to the neonate’s demands (25). Interestingly, in the absence of Stat5a, Stat5b is upregulated, and serial pregnancies increase its expression. Even though Stat5b is mainly involved in mediating the biological effects of growth hormone (GH) in the muscle and liver, it may substitute for Stat5a, to some extent, in inducing lactogenic processes (26). Taken together, accurate and controlled Stat5 activity is essential to adapting the most prominent properties of the mammary gland to the various stages of the reproductive cycle.

Deregulated Stat5 expression and activity induce mammary tumorigenesis

Despite its multifaceted activity, until a few years ago Stat5 was not considered a tumorigenic agent in the mammary gland. Among the Stat family members, breast cancer was associated with activated Stat3, which shares chromosomal localization with Stat5 but is mainly involved in the involution process of the gland. Stat3 is a pro-apoptotic agent in the postlactating mammary gland (27) and an essential mediator of the first phase of involution. It regulates apoptosis by inducing expression of distinct phosphatidyl-inositol 3-kinase (PI3K) regulatory subunits to downregulate PI3K/Akt-mediated survival signaling (28,29) and by controlling lysosomal-mediated cell death (30). Paradoxically, constitutive activity of Stat3 has been detected in human breast cancer cell lines and tumors (31–33) and programmed breast cancer cell death was induced by a dominant negative variant of the molecule (34). Collectively, these observations focused attention on Stat3 and the involution process as a main mediator of mammary/breast carcinogenesis.

Stat5 was detected in the nuclei of cells in 76% of breast cancers analyzed (35). In retrospect, the initial lack of a clear association between Stat5 activity and breast cancer development may have reflected the absence, at the time, of an appropriate model that would enable distinguishing the outcome of specific deregulated Stat5 activity from that of its activating factors. Prolactin activates Stat5 via Jak2. However, prolactin’s tumorigenic effect (36) may involve the Ras–mitogen-activated protein kinase signaling pathway (37), as well as additional kinase cascades, such as Src, the PI3K/Akt pathway (38) and Vav2-Neck3 (39). Jak2 is also a target of the protein tyrosine phosphatase 1B (40). Thus, initial tests for the tumorigenic effect of activated Stat5a in the mouse mammary gland were performed indirectly using WAP (whey acidic protein)-TGFlt (transforming growth factor α)-transgenic mice. These mice developed mammary tumors with a distinct histology and latency. When crossed with Stat5a−/− mice, the resulting genically modified Stat5a−/−/WAP (whey acidic protein)-TGFlt (transforming growth factor α) mice showed delayed induction of tumor formation (41), suggesting that Stat5 enhances the progression of TGFlt-mediated mammary tumorigenesis and might aid in the survival of tumor cells. In a second indirect tumor model, hemizygous Stat5a−/− mice developed the appearance of SV40-T antigen-mediated tumors. Decreased tumor size and increased apoptotic index in the cells of the developing adenocarcinomas were also noted (42).

Evidence for Stat5 involvement in mammary tumorigenesis was further substantiated in transgenic mice overexpressing the native STAT5 and its constitutively active variant, STAT5ca, which were directed for expression in the mammary gland of transgenic mice by regulatory sequences of the milk-protein β-lactoglobulin (BLG) gene. Overexpression and forced activation of STAT5 resulted in mammary tumors (43). An average incidence rate of ~10% was observed among the multistrain population and a record of 22% susceptibility was determined for an individual BLG/STAT5ca-transgenic line after a latency period of about 13 months. Indeed, by the time tumors were palpable, females had already reached the estrous phase, the equivalent of human menopause. Surprisingly, tumors also developed in strains of transgenic mice expressing the truncated STAT5 gene lacking its TAD. The ability of the various forms of Stat5—the trans-activating form and the one lacking its TAD—to induce tumorigenesis indicates that there is more than one mechanism by which Stat5 conveys its oncogenic role. This notion is supported by the resulting distinct tumor phenotypes: poorly differentiated carcinoma characterizes most of the tumors induced by the truncated form of Stat5, whereas highly differentiated papillary and micropapillary adenocarcinomas mainly result from overexpression and forced activation of Stat5. These phenotypes maintain a specific gene-expression signature (44) and array analysis demonstrated that the differences in gene expression between carcinomas and papillary adenocarcinomas funnel to distinct activation of at least six metabolic pathways involved in maintaining the different tumor phenotypes (44). Consequently, cell adhesion, motility and proliferation are favored in the carcinomas, whereas cell–cell contact, polarity, earlier cell-cycle arrest and DNA-damage response characterize the papillary adenocarcinomas. A nearly normal karyotype was displayed in cells originated from the papillary and micropapillary adenocarcinomas, whereas aneuploidies were found in the cell lines originating from tumors of the carcinomas (43). It may be stated, therefore, that protein interactions via the Stat5 TAD are necessary for the initiation of a differentiated and, in general, less-aggressive tumor phenotype, which may affect prognosis.

Importantly, the variants of Stat5 per se have phenotype-independent effects on gene expression in the tumors, resulting in a verified gene signature (45). Fifty genes were specifically affected by STAT5ca expression, and 94% of these were downregulated; the latter were involved in suppression of tumor suppressors and proliferation of antigonistics. This substantial downregulation distinguished the STAT5ca-induced tumorigenic consequences from the relatively equal effect of the C-terminally truncated Stat5 on gene expression, which included significant elevation in the expression of oncogenes and growth mediators such as Met and insulin-like growth factor II. Of note, very low levels of transgenic Stat5 variants were detected in the tumors. It is probably safe to assume that once initiated, tumor development in transgenic mice involves interaction of other groups of proteins (45). Nevertheless, the tumor inducer (i.e. deregulated Stat5 activity) can be tracked.

Parity-dependent Stat5 tumorigenic effect versus parity protection

Stat5’s tumorigenic effect is strictly dependent on preceding parity cycles. Deregulated Stat5 activity caused tumors almost exclusively in females that had undergone cycles of pregnancy and lactation, whereas their transgenic virgin counterparts were essentially protected (46). Pregnancy was also a prerequisite for tumor development in reconstituted glands, formed by transplantation of lenti-transfected epithelial cells expressing the constitutively active variant of Stat5, cSSF, into the cleared mammary fat pad of immunocompromised mice (47). Within 4 months, adenocarcinomas developed solely during involution, confirming the protective effect of the pregnancy hormones during the fertile period and the type of differentiated tumors initiated by deregulated Stat5 activity. The development of tumors from the transplanted cSSF-transformed cells also negates speculations on the involvement of pituitary abnormalities in Stat5-induced tumorigenesis in transgenic mice (48,49).
The offensive tumorigenic effect of Stat5, which strictly depends on parity, contrasts with the established protective effect of parity against breast cancer and intuitively diverges from the favorable prognosis reported for tumors with Stat5 nuclear localization. To reconcile these conflicts, the basis of parity’s protective effect is reviewed.

Epidemiological studies have established a negative correlation between parity and the incidence of breast cancer in women (50–52). Parity has a protective effect when full-term pregnancy occurs at an early age and this effect is enhanced by additional pregnancies (53,54). A less-obvious protective effect was recorded in parous women against development of ER/PR-negative tumors (55) and first pregnancy in women in their late 30s is believed to increase the relative risk of tumor development (51).

Similar to humans, both mice and rats exhibit parity-dependent protection against mammary cancer and serve as reliable models to study the mechanisms involved (52,56). In these models, administration of progesterone and estrogen can mimic the protective effects of pregnancy. Extensive efforts have been invested in revealing the cellular and molecular mechanisms involved in the parity’s protective effect by studying humans (50,57) and rodent models (58,59). Three main directions have been pursued: (i) differentiation and anti-proliferative effects of steroid and lactogenic hormones and (ii) permanent alterations in the expression of genes involved in the suppression of tumorigenesis. Exceptions and reservations accompany many of the results reported below. They mirror the incomplete protective effect of parity and imply a mutual contribution of multiple mechanisms whose dominance is altered in individual cases.

Parity induces differentiation and antiproliferative effects in the mammary gland

Differentiation of mammary epithelial cells under the influence of sex steroids has been proposed to result in the removal of highly proliferative or transformation-prone cells (60). Accordingly, parous glands do not exhibit a proliferative response to carcinogenic challenge (61–63). However, complete differentiation of the epithelial cells does not represent an obligatory prerequisite for protection against mammary carcinogenesis (61,64–67). A decrease in the number of undifferentiated stem cells, a putative target for cell transformation, in the mammary gland of females that experienced pregnancy was observed when compared with their virgin counterparts (68). These ER/PR-positive cells are probably not the stem cells themselves, which lack expression of these steroid receptors (69), but might be their luminal progenitors—the cells of origin for BRCA1-associated breast cancer (70).

Systemic effects of parity

The protective effects of parity can be reproduced in model animals by administration of estrogen and progesterone. Endogenous levels of these hormones are induced during pregnancy. On the other hand, elevated lifetime estrogen exposure is a major risk factor for breast cancer (71,72). A decrease in the circulating levels of GH was reported in parous compared with virgin rats (73) and was associated with reduced susceptibility to tumorigenesis (74,75). Surprisingly, the decrease in GH levels did not mirror altered GH expression in the pituitary gland and circulating levels of insulin-like growth factor 1 were not affected. Rather, the decreased GH levels were associated with decreased levels of activated Stat5 in the mammary gland of the 4 month old rats being analyzed (73). Interestingly, the differences between parous and age-matched virgin females in pStat5 level were not reproduced in a cohort of estrousual female mice (46), suggesting a latent effect of Stat5 activity on mammary tumorigenesis.

Parity induces permanent alterations in the expression of genes involved in suppression of tumorigenesis

A cell-fate hypothesis was proposed, suggesting that the pregnancy hormones estrogen and progesterone impose persistent changes in gene expression, which result in immediate blockage of carcinogenic-induced proliferation of ER-positive cells, possibly by abolishing the association between ER expression/localization and cell proliferation (76). Further studies in laboratory animals showed that, indeed, the parous glands do not exhibit a proliferative response after carcinogenic challenge (61–63) and that persistent upregulation of specific genes involved in epithelial differentiation, immune regulation and TGF signaling occurs. Downregulation of growth-related genes, such as Areg, Reg3a, Msln, Cdc2a, Igf2, Igfbp4, Smn1 and Msx1, was also noted (77). Moreover, the first full-term pregnancy induces a specific gene-signature profile in the breast epithelium that is still identifiable in parous women postmenopause (78). This signature characterizes, at the molecular level, the fully differentiated conditions that are associated with a reduction in breast cancer risk. Of note is the significant upregulation in the expression of seven DNA-damage response genes, including Rad51d and Xrc5d, suggesting better repair of the injured genome in the parous gland.

Potential routes by which deregulated Stat5 activity penetrates parity protection against breast cancer

Stat5 activity is regulated by parity cycles. It is induced during pregnancy and declines upon involution. How does deregulated Stat5 activity penetrate parity’s protective mechanisms to initiate mammary tumorigenesis?

The potential to overcome parity protection involves augmentation of the reduced GH signal in the parous gland by elevated Stat5 activity (Figure 1). In addition, higher Stat5 activity may increase the number of luminal progenitor cells in the mammary gland (79). This unique Stat5-dependent subpopulation is not subjected to the indirect signaling of steroid hormones in mid-late pregnancy shown for stem cells (80) and is highly prone to cancer (70). The potential effect of deregulated Stat5 activity on increasing progenitor cell number counteracts the parity-induced decrease in the number of stem/progenitor cells reported by Russo et al. (68) and the decrease in the number of stem cells in early pregnancy (81). In this context, the elimination of highly proliferative and cancer-prone cells by differentiation may be counteracted by the induced proliferative effect shown for the constitutively active Stat5 during pregnancy (24). Of interest are the opposing characteristics of the genomic signatures left by parity and by the constitutively active Stat5, STAT5ca, in the developing tumors. The parity signature mirrors activation of tumor suppressors such as the Myc suppressor Mnt. In contrast, expression of most of the genes that are specifically associated with higher Stat5 activity is downregulated in the tumors and many of these genes code for suppressors of tumor suppressors and proliferation antagonistic (45). No individual gene that was inversely affected by parity and deregulated Stat5 activity could be identified. This indicates differences in the specific mechanisms activating the opposing processes.

It has been suggested that the protective effect of parity is also mediated by chromatin structure/activators (82). Two markers of parity-related protection, RbAp46 and the non-coding RNA G.B7, have been implicated in a number of complexes involving chromatin remodeling and are persistently upregulated in the lobules of the regressed glands (83,84). An opposite consequence for the parity-mediated effect on chromatin was noted in the mammary gland of STAT5ca multiparous transgenic mice. Parity caused alterations in chromatin composition, specifically at the Stat5 binding sites in the promoters of cyclin D1 and Bclx genes, and individual cells with high levels of Stat5 nuclear localization were prone to cyclin D1-mediated tumorigenesis (46,85,86).

DNA damage is a major cause for cancer and activation of genes involved in the DNA-damage response is considered a protective mechanism in the parous glands (78). In contrast, forced activation of Stat5 induces oxidative stress during pregnancy, a period identified as vulnerable in this context, which causes DNA damage (87). Not all damaged cells complete the apoptotic process and die. Individual cells with high DNA damage and nuclear Stat5 localization were clearly located at the center of early hyperplasia, most probably inducing tumorigenesis (87).
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Finally, it should be noted that the undermining of parity protection by deregulated Stat5 activity may also occur in a parity-independent manner, for example by affecting the ER–Stat5 interaction. ERα is the key mediator of estrogen action in the intact mammary gland and its dysregulation is an important step in early tumorigenesis. Enhanced ERα expression sensitizes the premalignant epithelium to the proliferative effects of their cognate ligands (88). Stat5α has been reported to physically interact with ERα in the mammary epithelium (89) and its conditional repression resulted in immediate downregulation of ERα expression (25). Consequently, loss of Stat5 reduces the prevalence of ERα-initiated mammary preneoplasia (90). Taken together, these experiments strongly imply that higher expression of Stat5 will dictate elevated levels of ERα in individual cells, rendering the gland susceptible to cancer.

In summary, studies in animal models indicate that deregulated Stat5 levels and activity represent a genuine risk factor for breast cancer. The Stat5 tumorigenic effect is parity dependent and probably involves manipulation of parity’s protective effects. Tumorigenesis is induced during pregnancy when Stat5 exerts immediate hazardous effects on the total epithelial cell population. These effects are not phenotypically visible, probably due to the protective effect of steroid hormones. Latent tumor initiation is triggered only in individual cells. Identifying the rare population that initiates tumor development represents, therefore, a justifiable aim. An apparent and most probable characteristic of these cells is high nuclear Stat5 translocation—the same feature that marks better prognosis in the tumors. These tumors are mainly differentiated adenocarcinomas or their subtypes, which develop during the estrous or earlier, depending on the animal model.

From a clinical point of view, these data call for the introduction of safe methods for screening deregulated Stat5 activity during pregnancy as a protective measure against breast cancer development. Preferably, the development of specific and representative gene signatures that can be generated from a relatively small number of cells. Inhibitors of the Jak2/Stat5 pathway (91–93) and Stat5 activity (94,95) have been developed but their specific targeting to the breast-cell population is difficult and may decrease enthusiasm for their clinical application. For this reason, introducing an efficient small interfering RNA-delivery system (96) for tissue-specific Stat5 inhibitors constitutes a valid goal.

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