Virchows Arch (2006) 448: 165–171
DOI 10.1007/s00428-005-0056-6

ORIGINAL ARTICLE

Gary L. Bratthauer · Brian L. Strauss · Fattaneh A. Tavassoli

STAT 5a expression in various lesions of the breast

Received: 16 March 2005 / Accepted: 27 July 2005 / Published online: 19 August 2005

Abstract The seven signal transducer and activator of transcription (STAT) molecules are effectors of hormonal or cytokine stimulation through receptors. STAT 5a, isolated from prolactin-stimulated mammary cells, contributes to normal proliferation and is essential for mammary gland differentiation. Using a monoclonal antibody, we tested 100 formalin-fixed, paraffin-embedded breast tissues representing everything from simple hyperplasia to invasive carcinoma for the expression of STAT 5a in comparison to normal breast epithelial cells. Immunohistochemical analysis was performed following heat treatment in a pressure cooker. STAT 5a was found in endothelial cells, adipocytes, and leukocytes as well as in the cytoplasm and nucleus of normal epithelial cells, usual ductal hyperplasia, and benign lesions such as fibroadenoma. Myoepithelial cells and stromal fibroblasts failed to demonstrate any STAT 5a in addition to most atypical proliferations including in situ and invasive carcinomas. A few examples of lobular intraepithelial neoplasia and invasive carcinoma demonstrated some reactivity, albeit comparatively reduced. The absence of STAT 5a in the abnormal breast epithelial cells may indicate a defect contributory to the abnormal state.

Keywords Breast · Carcinoma · Immunohistochemistry · STAT 5a

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official, or as reflecting the views of the Department of the Army, Department of the Air Force, or Department of Defense.

G. L. Bratthauer (✉) · B. L. Strauss · F. A. Tavassoli
Department of Gynecologic and Breast Pathology,
Armed Forces Institute of Pathology,
Washington, DC 20306-6000, USA
e-mail: bratthauer@afip.osd.mil
Tel.: +1-202-7821600
Fax: +1-202-7823939

Present address:
B. L. Strauss
Department of Pathology, Wilford Hall Medical Center,
Lackland Air Force Base,
San Antonio, TX 78236, USA

Present address:
F. A. Tavassoli
Department of Pathology, Yale University School of Medicine,
New Haven, CT 06520-8023, USA

Introduction

The seven signal transducer and activator of transcription (STAT) molecules are effectors of hormonal or cytokine stimulation through receptors. In unstimulated cells, STAT proteins exist largely in the cytoplasm as latent transcription factors. In response to exogenous factors, specific STATs undergo tyrosine phosphorylation, nuclear translocation, and DNA binding resulting in the transcriptional activation of distant target genes. There are many genes regulated by the STAT pathway that are required for cell growth, survival, differentiation, and motility [7].

STAT 5a, also called mammary growth factor, was isolated from prolactin-stimulated mammary cells, and is activated through the binding of prolactin to its receptor [17]. STAT 5a has been shown to be involved in integrin-mediated c-*fos* transcription and integrin-mediated adhesion affects control of cell cycle entry and inhibition of apoptosis [5]. In knockout mice that lack STAT 5a, the mammary gland does not develop properly [1, 14]. STAT 5a-deficient mice develop normally, but lobuloalveolar growth during pregnancy is aberrant [15].

It has been suggested that STAT 5a contributes to normal proliferation, and it has been shown that STAT 5a is essential for mammary gland differentiation in mice [15]. Furthermore, known defects in the Janus Kinase (JAK)/STAT pathway have led to the inhibition of growth restraint [19]. STAT 5a is also involved in adipocyte differentiation, its expression correlating with lipid accumulation, and it can be activated in the stroma by growth hormone and the epidermal growth factor [9, 24]. Recently, STAT 5a activation in breast cancer has been associated with a favorable prognosis [18].

Using a monoclonal antibody, we immunohistochemically tested different examples of breast disease for the expression of STAT 5a in comparison to normal breast ductal and lobular epithelial cells.

Materials and methods

For this study, 100 formalin-fixed, paraffin-embedded tissues representing various lesions of the breast were obtained from the files of the Armed Forces Institute of Pathology. The sections were reviewed for the presence of normal breast ductal and lobular epithelium, low-risk ductal intraepithelial neoplasia (DIN) (usual ductal hyperplasia), DIN 1 (atypical ductal hyperplasia), lobular intraepithelial neoplasia (LIN) (atypical lobular hyperplasia or lobular carcinoma in situ), DIN 1–3 (ductal carcinoma in situ), and invasive carcinoma [27]. Specimens were sectioned at 6 μm and then assayed with an antibody to STAT 5a (clone ST5a-2H2, Zymed Laboratories, South San Francisco, CA). Immunohistochemical analysis was performed as previously described [4]. Briefly, formalin-fixed, paraffin-embedded sections were heated at 70°C for 30 min and placed into a prewarmed pressure cooker in a solution of Reveal (Biocare Medical, Concord, CA) in which deparaffinization and antigen retrieval were performed simultaneously at 20 lb/in.^{2} for 3 min. Following depressurization, sections were placed into a prewarmed fresh solution of Reveal for 30 min before being rinsed in warm water. Endogenous peroxide and oxidative compounds were quenched by incubation in 5% H_2O_2 in methanol. Sections were rehydrated in Tris-buffered saline (TBS Plus, Biocare Medical) with 0.1% Tween 20 (DakoCytomation, Carpinteria, CA) added, prior to antibody application. Primary antibody incubations were performed for 60 min, with the ST5a-2H2 antibody diluted in TBS–Tween at 1:400. Detection reagents (ABC Universal Elite, Vector Laboratories Inc., Burlingame, CA) were applied for 45 min each, and all dilutions and intervening rinses were with TBS–Tween. Diaminobenzidine tetrahydrochloride (Sigma Chemical Co., St. Louis, MO) (0.08%) with 0.025% H_2O_2 added in a solution of TBS–Tween (DAB) served as the chromogenic substrate. Sections were allowed to develop in DAB for 12 min, counterstained with hematoxylin, dehydrated, and coverslipped with Permount (Fisher Chemical Co., Pittsburgh, PA) in xylene.

Assayed sections were examined for accurate interpretation of positive signal on a qualitative intensity scale. Tissues were regarded as positively expressing STAT 5a when qualitative intensity was at least 2 on a 4-point scale, and more than 20% of the cells were reactive. The cell type along with reaction product distribution was noted.

Results

The results are listed in Table 1. In normal mammary gland epithelium, STAT 5a was found to exist in the cytoplasm and the nucleus of about 80% of the luminal breast epithelial cells in the lobules and larger ducts (Fig. 1). Myoepithelial cells failed to demonstrate any STAT 5a, and stromal cell fibroblasts were also nonreactive. STAT 5a was, as expected, found in endothelial cells, adipocytes, and leukocytes [2, 12, 29].

Table 1 STAT 5a reactivity in various examples of breast epithelium

Histology	Total no. of cases	STAT 5a+no. of cases	%Positive
Normal/UDH	100	100[a]	100
Fibroadenoma	2	2	100
ADH (DIN 1)	14	0	0
LIN (ALH/LCIS)	25	8[b]	32
DCIS (DIN 1–3)	25	1[b]	4
Invasive Ca	30	5[c]	17

UDH Usual ductal hyperplasia, *ADH* Atypical ductal hyperplasia, *LIN* Lobular intraepithelial neoplasia, *ALH* Atypical lobular hyperplasia, *LCIS* Lobular carcinoma in situ, *DCIS* Ductal carcinoma in situ, *DIN* Ductal intraepithelial neoplasia

[a] Positive in 80% or more of cells

[b] Reactive but less so than surrounding normal cells

[c] Two tubular carcinomas and three invasive carcinomas with squamoid features and inflammation showed focally positive immunostaining

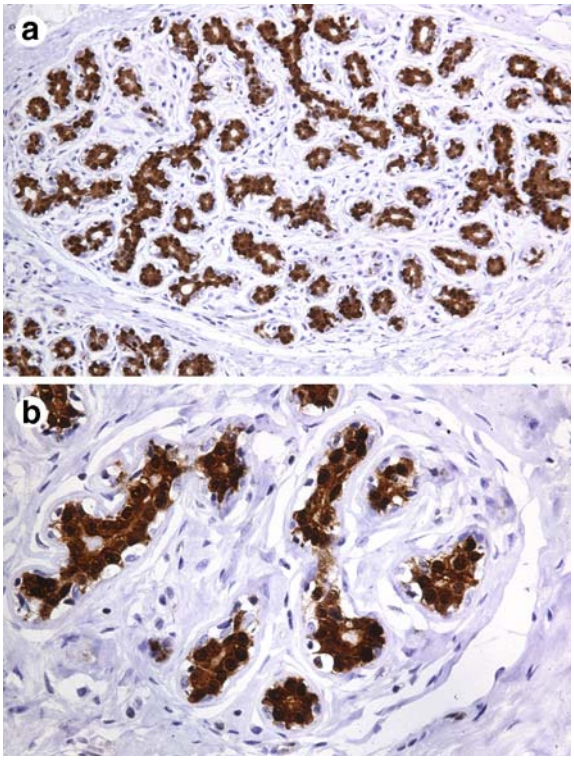

Fig. 1 **a** A normal breast lobule showing positive decoration for the presence of STAT 5a by immunohistochemistry (200×). **b** Normal breast lobule, immunohistochemical demonstration of STAT 5a in the cytoplasm and nucleus (400×)

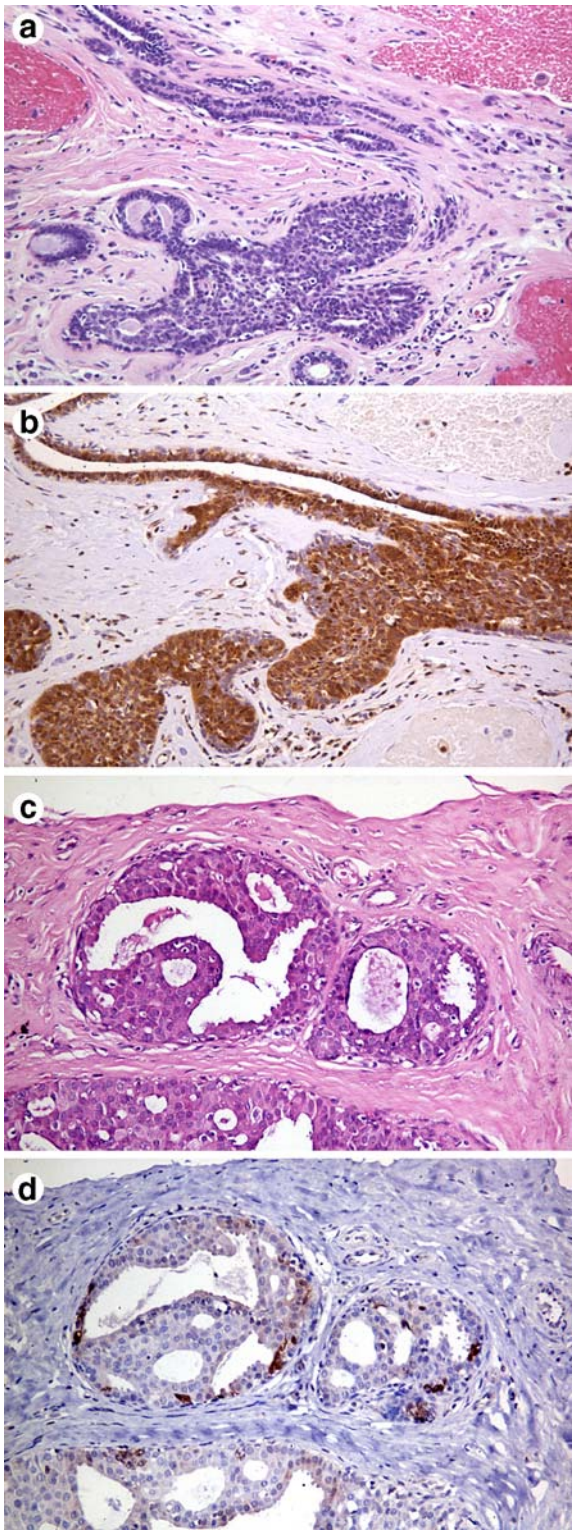

Fig. 2 **a** Hematoxylin and eosin (H&E) stain of a focus of low-risk DIN (usual ductal hyperplasia) (200×). **b** Immunohistochemical demonstration of STAT 5a in low-risk DIN (usual ductal hyperplasia) (200×). **c** H&E stain of a focus of DIN 1 (atypical ductal hyperplasia) (200×). **d** Immunohistochemical demonstration of the absence of STAT 5a in DIN 1 (atypical ductal hyperplasia) (200×). Note: scattered residual STAT 5a-positive normal epithelial cells

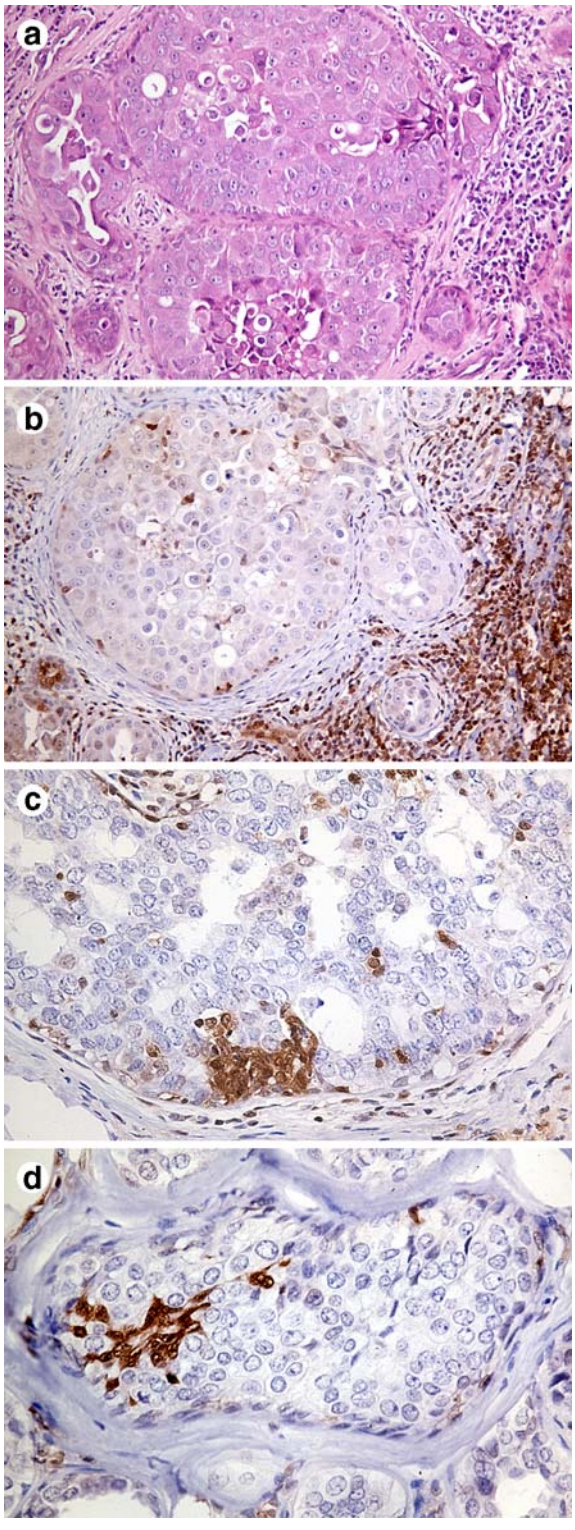

Fig. 3 **a** H&E stain of a focus of DIN 3 (ductal carcinoma in situ, grade 3) (200×). **b** Immunohistochemical demonstration of the absence of STAT 5a in the carcinoma (200×). Note: STAT 5a-positive inflammatory cells. **c**,**d** Immunohistochemical demonstration of the absence of STAT 5a in DIN 1 (ductal carcinoma in situ, grade 1) (400×). Note: residual STAT 5a-positive normal epithelial cells

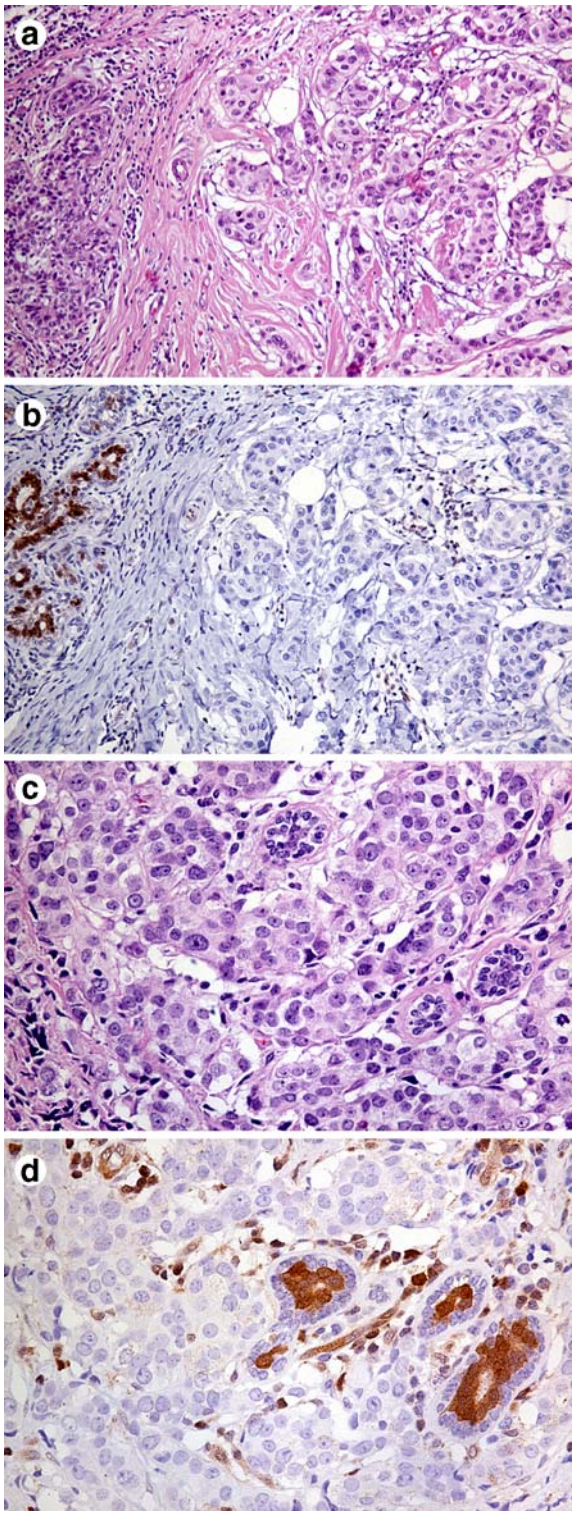

Fig. 4 **a** H&E stain of invasive ductal carcinoma (200×). **b** Immunohistochemical demonstration of the absence of STAT 5a in the carcinoma (200×). Note: adjacent normal STAT 5a-positive epithelial cells. **c** H&E stain of invasive ductal carcinoma (400×). **d** Immunohistochemical demonstration of the absence of STAT 5a in the carcinoma (400×). Note: entrapped normal STAT 5a-positive epithelial cells

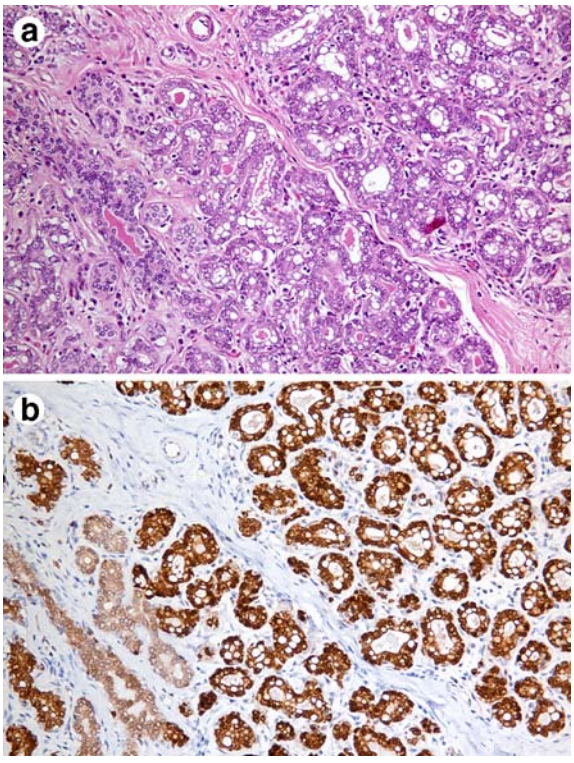

Fig. 5 **a** H&E stain of a focus of epithelial cell secretory change (200×). **b** Immunohistochemical demonstration of STAT 5a in the cells with secretory changes (200×). Note: intensity greater than that seen in neighboring epithelial cells

STAT 5a was detected in the cytoplasm and nucleus of epithelial cells in cases of usual ductal hyperplasia and in benign neoplasms such as fibroadenoma. However, in atypical proliferations, ductal carcinoma in situ (DIN 1–3) and in invasive carcinomas, the STAT 5a protein (with rare exceptions) was either not expressed or greatly reduced (Figs. 2, 3 and 4). It was absent in all cells with apocrine differentiation but was readily detected in cells undergoing secretory or lactational change. The reactivity in the secretory cells was often more intense than that seen in the surrounding normal cells, perhaps signaling a higher concentration of antigen in these cells (Fig. 5). Although most examples of LIN did not express STAT 5a, a few examples demonstrated some reduced reactivity. Also, two tubular carcinomas and three invasive carcinomas with squamoid features and inflammation exhibited focal reactivity (data not shown). This may be secondary to increased background STAT 5a in these cases, since it occurred only when the adjacent normal epithelial and inflammatory cells also showed increased STAT 5a expression as compared to STAT 5a-negative carcinomas. All control sections assayed with a normal mouse IgG in substitution for the STAT 5a antibody were nonreactive.

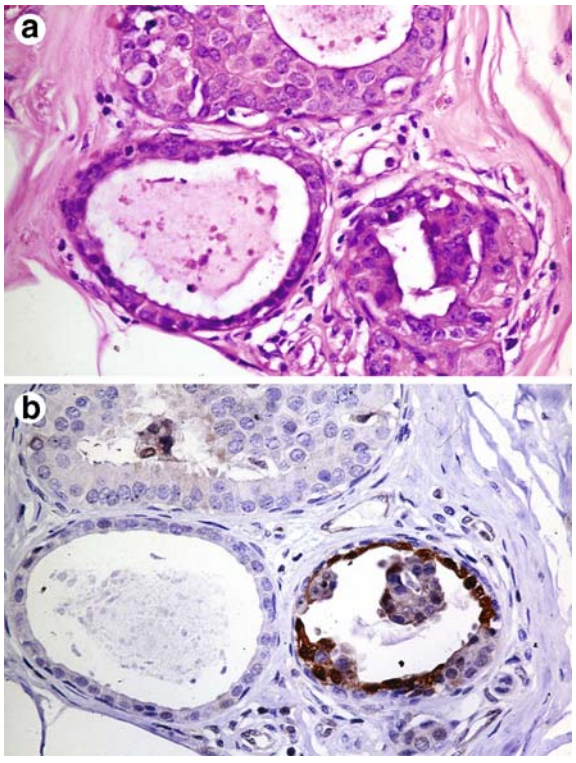

Fig. 6 **a** H&E stain showing small foci of DIN 1 Flat type (flat type epithelial atypia) and DIN 1 (atypical ductal hyperplasia) (400×). **b** Immunohistochemical detection of STAT 5a demonstrating its absence in these abnormal foci (400×)

Discussion

A reduction of STAT 5a was observed, associated with abnormal breast epithelial cells. The absence of STAT 5a in the abnormal breast epithelial cells may indicate a defect contributory to the abnormal state. Since STAT 5a is required for the development of breast lobules, repressors of its activation or inappropriate signaling through other mechanisms might alter the function and phenotype of these cells. Because the demonstrated expression was seen specifically in cells known from other studies to contain STAT 5a, it was reasoned that the reaction was specific for STAT 5a or a STAT 5a-like protein. In all of the sections, normal ductal and lobular epithelium reacted strongly in the cytoplasm and nucleus with the antibody to STAT 5a, serving as an internal control for cells with weak or absent staining.

STAT 5a expression may be affected by an abnormal genotype. Certain genomic deletions may affect the STAT 5a gene locus, found on chromosome 17q11, incidentally. However, it is also possible that any number of biochemical events involving STAT 5a may act to provide an atmosphere in which cellular transformation can occur. The absence of STAT 5a could result in aberrant signaling or suboptimal transcription of developmental, regulatory, or growth-restraining proteins contributing to altered (metaplastic or neoplastic) phenotypic differentiation.

Numerous reports implicate the suppression of STAT 5a in normal and disease processes [20, 22, 24]. Suppressors of cytokine signaling (SOCS) and protein inhibitors of activated STATs (PIAS) can suppress signal transduction by inhibiting the STAT pathway [10, 26, 28]. Activated SOCS can also stimulate cell proliferation and increase cell survival through the Ras signaling pathway [6]. Therefore, proliferation can occur through at least one mechanism that would inactivate STAT 5a in the process and, in fact, a recent study demonstrated increased SOCS gene expression in breast cancer [21].

The absence of STAT 5a in most abnormal breast epithelial cells including carcinomas is not something that has been widely noted, however. Because STAT 5a is a transcription factor, one might imagine that an over-expression or constitutive expression of STAT 5a would be seen in the uncontrolled growth of breast carcinomas like the reports of over-expression of STAT 3 in these tumors [3]. This has been reported in one study where 76% of the breast cancers examined showed phosphorylated STAT 5a to be present in the nucleus [8]. It has also been shown that in 65% of induced rat mammary gland tumors, STAT 5a was detected in the nucleus using phosphorylation-specific antibodies [23].

These data may seem, initially, to contradict the present findings. Antibody variability, however, can certainly make a difference in detection. Nevalainen et al. saw a decrease in activated STAT 5a in more aggressive breast cancers using a phosphor-specific antibody, while detection of the STAT 5a protein using another anti-STAT 5a was maintained [18]. In contrast, the report by Shan et al. [23] stated that high STAT 5a nuclear expression closely correlated with higher-grade carcinomas and identified a cytoplasmic location for STAT 5a in the normal rat mammary gland. Because the STAT 5a expression in this present study was seen in the nucleus, it was reasoned that at least some of the identified STAT 5a might be of an activated form. The ST5a-2H2 clone used for this study, while not described as an activation specific clone, demonstrated a lack of detection of STAT 5a in higher-grade lesions corroborating the results of Nevalainen et al. [18].

The ST5a-2H2 antibody is directed against the molecule's carboxy-terminus near where the active phosphorylation site is located. Some functional STAT 5a variants may exist that are not detectable with this antibody, and some naturally occurring splice site variants of STAT 5a have been shown to have the ability to suppress the transcriptional activity of the wild-type STAT 5a [30]. Also, a few early studies have pointed to distinct carboxy-terminal truncated forms of STAT 5a [13, 16]. If truncated forms were present in the tumor cells, they would not be visible with this antibody. Tantalizingly, a report examining the expression of various forms of STAT 5a in transgenic mice demonstrated that a carboxy-terminal truncated form of STAT 5a produced the most undifferentiated carcinomas in the mammary glands of these mice [11].

It was of interest to note that a relative over-expression of STAT 5a was observed in cells undergoing secretory change. This was considered unusual because cells under-

going apocrine differentiation (a secretory cell) were uniformly nonreactive. Perhaps the secretory nature of breast epithelium is dependent on viable STAT 5a, and the functional ability of the cell is lost when defects occur in this pathway leading to abnormal cell behavior. Also, the rare lesions showing some expression were either well-differentiated tubular carcinomas or tumors surrounded by a large amount of STAT 5a-positive inflammatory cells. Examples of LIN (merely an indicator of generalized risk) demonstrated the expression nearest to that of normal or hyperplastic samples, and in the study by Cotarla et al., the STAT 5a-positive tumors correlated with increased levels of differentiation [8]. This may indicate that STAT 5a function is maintained in lesions that are differentiated enough to allow normal processes to occur. These findings support STAT 5a as a marker of good prognosis, and the percentage of positive carcinomas reported by Nevalainen et al. is also similar to the number reported here [18]. In addition, a recent report described STAT 5a as a suppressor of invasion due to its loss associated with metastatic lesions and a relationship between STAT 5a and adhesion [25]. STAT 5a, then, has been alternately reported to be an active factor associated with breast carcinoma or acting almost like a tumor suppressor gene in the breast. The correlation of function and tumor grade with respect to STAT 5a expression as determined by the individual antibody employed will have to be investigated further.

Whether or not STAT 5a has an effect on the maintenance of the normal phenotype as it has an effect on the establishment of the normal phenotype is subject to conjecture. In most instances, abnormal cell growth can be identified by an absence of STAT 5a reactivity (Fig. 6). The St5a-2H2 antibody, therefore, might also have a possible clinical utility in distinguishing atypical ductal hyperplasia or grade one ductal carcinoma in situ (DIN 1) from usual ductal hyperplasia (low-risk DIN). Since STAT 5a is required for the normal development of breast lobules, repressors of its activation or inappropriate signaling through other mechanisms might alter the function and phenotype of breast epithelial cells.

Acknowledgements The authors gratefully acknowledge Mr. Doug Landry for the production of the figure files.

References

1. Akira S (1999) Functional roles of STAT family proteins: lessons from knockout mice. Stem Cells 17:138–146
2. Balhoff JP, Stephens JM (1998) Highly specific and quantitative activation of STATs in 3T3-L1 adipocytes. Biochem Biophys Res Commun 247(3):894–900
3. Berclaz G, Altermatt HJ, Rohrbach V, Siragusa A, Dreher E, Smith PD (2001) EGFR dependent expression of STAT 3 (but not STAT 1) in breast cancer. Int J Oncol 19(6):1155–1160
4. Bratthauer GL (1999) The avidin–biotin complex (ABC) method and other avidin–biotin binding methods. In: Javois LC (ed) Immunocytochemical methods and protocols, 2nd ed. Methods Mol Biol 115. Humana, Totowa, NJ, pp 203–214
5. Brizzi MF, Defillippi P, Rosso A, Venturino M, Garbarino G, Miyajima A, Silengo L, Tarone G, Pegoraro L (1999) Integrin-mediated adhesion of endothelial cells induces JAK2 and STAT 5a activation: role in the control of c-fos gene expression. Mol Biol Cell 10:3463–3471
6. Cacalano NA, Sanden D, Johnston JA (2001) Tyrosine-phosphorylated SOCS-3 inhibits STAT activation but binds to p120 RasGAP and activates Ras. Nat Cell Biol 3:460–465
7. Clevenger CV (2004) Roles and regulation of STAT family transcription factors in human breast cancer. Am J Pathol 165:1449–1460
8. Cotarla I, Ren S, Zhang Y, Gehan E, Singh B, Furth PA (2004) Stat5a is tyrosine phosphorylated and nuclear localized in a high proportion of human breast cancers. Int J Cancer 108 (5):665–671
9. Gallego MI, Binart N, Robinson GW, Okagaki R, Coschigano KT, Perry J, Kopchick JJ, Oka T, Kelly PA, Hennighausen L (2001) Prolactin, growth hormone, and epidermal growth factor activate STAT 5a in different compartments of mammary tissue and exert different and overlapping developmental effects. Dev Biol 229:163–175
10. Greenhalgh CJ, Hilton DJ (2001) Negative regulation of cytokine signaling. J Leukoc Biol 70:348–356
11. Iavnilovitch E, Cardiff RD, Groner B, Barash I (2004) Deregulation of Stat5 expression and activation causes mammary tumors in transgenic mice. Int J Cancer 112(4):607
12. Kagami S, Nakajima H, Suto A, Hirose K, Suzuki K, Morita S, Kato I, Sato Y, Kitamura T, Iwamoto I (2001) STAT 5a regulates T helper cell differentiation by several distinct mechanisms. Blood 97(8) 2358–2365
13. Kazansky AV, Raught B, Lindsey SM, Wang YF, Rosen JM (1995) Regulation of mammary gland factor/Stat5a during mammary gland development. Mol Endocrinol 9(11):1598–1609
14. Levy DE (1999) Physiological significance of STAT proteins: investigations through gene disruption in vivo. Cell Mol Life Sci 55:1559–1567
15. Miyoshi K, Shillingford JM, Smith GH, Grimm SL, Wagner KU, Oka T, Rosen JM, Robinson GW, Hennighausen L (2001) Signal transducer and activator of transcription (STAT) 5 controls the proliferation and differentiation of mammary alveolar epithelium. J Cell Biol 155:531–542
16. Mui AL, Wakao H, Harada N, O'Farrell AM, Miyajima A (1995) Interleukin-3, granulocyte-macrophage colony-stimulating factor, and interleukin-5 transduce signals through two forms of STAT5. J Leukoc Biol 57(5):799–803
17. Nevalainen MT, Xie J, Bubendorf L, Wagner KU, Rui H (2002) Basal activation of transcription factor signal transducer and activator of transcription (STAT 5) in nonpregnant mouse and human breast epithelium. Mol Endocrinol 16:1108–1124
18. Nevalainen MT, Xie J, Torhorst J, Bubendorf L, Haas P, Kononen J, Sauter G, Hallgeir R (2004) Signal transducer and activator of transcription-5 activation and breast cancer prognosis. J Clin Oncol 22(11):2053–2060
19. Pansky A, Hildebrand P, Fasler-Kan E, Baselgia L, Ketterer S, Beglinge C, Heim MH (2001) Defective Jak-STAT signal transduction pathway in melanoma cells resistant to growth inhibition by interferon alpha. Int J Cancer 85:720–725
20. Paukku K, Valgeirsdettir S, Saharinen P, Bergman M, Heldin CH, Silvennoinen O (2000) Platelet-derived growth factor (PDGF)-induced activation of signal transducer and activator of transcription (STAT) 5 is mediated by PDGF beta-receptor and is not dependent on c-Src, Fyn, Jak1 or Jak2. Biochem J 345:759–766
21. Raccurt M, Tam SP, Lau P, Mertani HC, Lambert A, Garcia-Caballero T, Li H, Brown RJ, McGuckin MA, Morel G, Waters MJ (2003) Suppressor of cytokine signalling gene expression is elevated in breast carcinoma. Br J Cancer 89(3):524–532
22. Schroeder MD, Rose-Hellekant TA, Sandgren EP, Schuler LA (2001) Dysregulation of mammary Stats 1, 3 and 5 and PRL receptors by overexpresssion of TGF-alpha. Mol Cell Endocrinol 175(1–2):173–183

23. Shan L, Yu M, Clark BD, Snyderwine EG (2004) Possible role of Stat5a in rat mammary gland carcinogenesis. Breast Cancer Res Treat 88(3):263–272
24. Stewart WC, Morrison RF, Young SL, Stephens JM (1999) Regulation of signal transducers and activators of transcription (STATs) by effectors of adipogenesis: coordinate regulation of STATs 1, 5a, and 5b with peroxisome proliferator-activated-receptor-gamma and C/AAAT enhancer binding protein-alpha. Biochim Biophys Acta 1452:188–196
25. Sultan AS, Xie J, LeBaron MJ, Ealley EL, Nevalainen MT, Rui H (2005) Stat5 promotes homotypic adhesion and inhibits invasive characteristics of human breast cancer cells. Oncogene 24:746–760
26. Tam SP, Lau P, Djiane J, Hilton DJ, Waters MJ (2001) Tissue-specific induction of SOCS gene expression by PRL. Endocrinology 142:5015–5026
27. Tavassoli FA (2005) Breast pathology: rationale for adopting the ductal intraepithelial neoplasia (DIN) classification. Nat Clin Pract Oncol 2:116–117
28. Tomic S, Chughtai N, Ali S (1999) SOCS-1, -2, -3: selective targets and functions downstream of the prolactin receptor. Mol Cell Endocrinol 158:45–54
29. Yahata Y, Shirakata Y, Tokumaru S, Yamasaki K, Sayama K, Hanakawa Y, Detmar M, Hashimoto K (2003) Nuclear translocation of phosphorylated STAT 3 is essential for vascular endothelial growth factor-induced human dermal microvascular endothelial cell migration and tube formation. J Biol Chem 278 (41):40026–40031
30. Yamashita H, Iwase H, Toyama T, Fujii Y (2003) Naturally occurring dominant-negative STAT 5 suppresses transcriptional activity of estrogen receptors and induces apoptosis in T47D breast cancer cells. Oncogene 22(11):1638–1652