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Effects of hyperactive Janus kinase 2 signaling in mammary epithelial cells

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Abstract

Prolactin, the Janus kinase 2 (JAK2) and the signal transducer and activator of transcription 5 (STAT5) are important for mammary gland development and have also been implicated in development and growth of breast tumors. In the present study we have investigated the role for JAK2 in proliferation, differentiation, and apoptosis of the mammary epithelial cell line HC11 by stably overexpressing two hyperactive JAK2 mutants. Cells expressing a JAK2 mutant consisting of only the kinase domain had high amount of nuclear STAT5 protein with low DNA-binding activity, which was rapidly induced to a DNA-binding state by prolactin treatment. Cells expressing JAK2 deleted of the kinase-like domain showed increased sensitivity to prolactin treatment compared to wild type cells. Proliferation was not affected by any of the mutants whereas the ability to undergo apoptosis was decreased implicating a transforming potential of the JAK2 mutants.

Keywords: JAK2; STAT5; Mammary epithelial cells

The Janus kinases (JAK) constitute a cytoplasmic protein-tyrosine kinase family which, at present, has four members, JAK1-3 and Tyk2 [1]. These tyrosine kinases have been shown to be essential for cytokine signaling, as shown in JAK1 and JAK2 gene-deletion experiments [2–4]. Different cytokines activate different JAK kinases. Cytokine activation of JAK molecules has been shown to result in activation of several intracellular signaling pathways and, dependent on the type of cells studied, modulation of proliferation, differentiation, and apoptosis [5–7]. JAK2 is critical for proliferation and differentiation of mammary epithelium [8]. Several studies indicate that mutated, hyperactive JAK molecules have the capacity to transform cells [9]. The current model for activation of JAK is that ligand-induced receptor dimerization brings two receptor-associated JAK molecules in close proximity which in turn initiates trans-tyrosine phosphorylation and activation of the JAK molecules [10]. This activation model could also explain ligand-independent activation of JAK kinases in mammalian [11] and insect cells [12–14] overexpressing the molecule. Upon activation the receptor associated JAK phosphorylates tyrosine residues in the intracellular part of the receptor. These phosphorylated residues function as docking sites for SH2 domain containing adaptor molecules and signal mediators, which in turn can be phosphorylated by JAK. One intracellular target for JAK is the signal transducers and activators of transcription (STAT) family of proteins. To date seven mammalian STAT proteins have been described [15].

The pituitary peptide hormone prolactin (PRL) is an important regulator of mammary gland development, as shown in studies in which the gene for PRL or the prolactin receptor (PRLR) was deleted [16,17]. PRL has also been implicated in development and growth regulation of mammary gland tumors [18]. PRL activates different intracellular signaling pathways through activation of JAK2. In mammary epithelial cells PRL activates STAT5, originally called mammary gland factor [19]. STAT5 exists as two forms, STAT5A and

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STAT5B, which are transcribed by separate but highly homologous genes [20,21]. Deletion of these genes, separate or in combination, has been shown to result in impaired mammary gland development [22,23].

The mammary epithelial cell line HC11 has been shown to be a valuable tool in studies on molecular mechanisms involved in gene regulation and differentiation of the mammary gland [24]. By changing culture conditions molecular processes from growth phase to terminal differentiation can be studied. Aberrant activity of JAK kinase or STAT has been implicated in tumorigenesis. We therefore found it interesting to analyze the effects of overexpression of hyperactive JAK2 mutants on proliferation, differentiation, and apoptosis of HC11 cells. Increased nuclear STAT5 protein, which does not bind DNA in the absence of PRL, was found in HC11 cells expressing a JAK2 mutant consisting of only the kinase domain. Cells expressing a JAK2 mutant deleted of the kinase-like domain showed increased STAT5 activation after PRL treatment. HC11 cells expressing either JAK2 mutant showed decreased ability to undergo apoptosis.

Materials and methods

Cell culture and transfection. HC11 cells were grown in complete medium: RPMI 1640, L-glutamine, 50 μg/ml gentamycin, 10% fetal bovine serum (FBS), 10 ng/ml epidermal growth factor (Sigma), and 5 μg/ml insulin (Sigma). Confluent cells were washed and kept for 48 h in RPMI 1640, 2% FBS, and 5 μg/ml insulin, rendering them competent to stimuli of lactogenic hormones. Cells were then treated with 1 μg/ml ovine PRL (oPRL; Sigma) for different times (see legends to figures). For induction of β-casein expression in HC11 cells 0.1 μM dexamethasone (Sigma) was added together with oPRL. HC11 cells were transfected with 10 μg of pCI-neo-JAK2 constructs using the calcium phosphate precipitation technique, as previously described [25]. Stable clones were selected in 240 μg/ml geneticin.

Nuclear extract. Nuclear extracts were prepared using a modification of the method of Dignam et al. [26]. Cells were washed with cold PBS, and pelleted in a microcentrifuge. The cells were resuspended in RSB buffer (10 mM Tris, pH 7.4, 10 mM NaCl, and 6 mM $MgCl_2$), kept on ice for 5 min, and spun down again. The cell pellet was resuspended in RSB containing 1 mM DTT, 0.4 mM PMSF, and 0.1 mM Na_3VO_4 and homogenized by 30 strokes in a glass homogenizer. After centrifugation, the nuclear pellet was resuspended in 3 volumes of buffer C (20% glycerol, 20 mM Hepes, pH 7.9, 420 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 1 mM DTT, 0.2 mM PMSF, and 0.5 mM Na_3VO_4) and kept on ice for 20–30 min. The supernatant obtained after centrifugation was used as nuclear extract.

Electrophoretic mobility shift assay (EMSA). Nuclear extracts were incubated with 167 μg/ml poly(dI/dC) for 10 min at room temperature in EMSA buffer (12 mM Hepes, pH 7.9, 4 mM Tris, pH 7.0, 0.1 mM EDTA, 1 mM DTT, and 4% Ficoll). Fifty thousand cpm of ^{32}P-labeled β-casein probe (TGCTTCTTGGAATT) was added and incubated for an additional 10 min. Specific binding to the probe was analyzed on a 4.5% polyacrylamide gel, prerun for 2 h at 250 V in 0.25X TBE (22.5 mM Tris borate, pH 8.0, 0.5 mM EDTA). The samples were loaded and electrophoresed for 1 h at 250 V, after which the gel was dried and exposed to X-ray film.

Whole cell extract. Cells were washed with cold PBS, harvested in lysis buffer (1% NP40, 50 mM Tris–HCl, pH 7.5, 140 mM NaCl, 2 mM EDTA, 10 μg/ml leupeptin, 10 μg/ml pepstatin, 0.2 U/ml aprotinin, 1 mM PMSF, and 1 mM Na_3VO_4 and kept on ice for 20 min. The cell lysates were thereafter clarified by centrifugation at 4 °C for 10 min in a microcentrifuge.

Western blotting. Proteins from nuclear or whole cell extracts were separated on a 7.5% or 12% SDS–PAGE gel, for detection of STAT5 and β-casein, respectively. Separated proteins were transferred to a PVDF membrane by semidry blotting. The membrane was blocked for 1 h (or overnight) in TBS (20 mM Tris–HCl, pH 7.5, 150 mM NaCl) containing 5% (w/v) milk protein (STAT5) or 5% (w/v) BSA (β-casein). After washing, the membrane was incubated for 1 h with mouse anti-STAT5 (Transduction Laboratories; diluted 1:250) or rabbit anti-β-casein (a kind gift from Dr. E Reichmann, Institut Suisse de Recherches Experimentales sur le Cancer, Lausanne, Switzerland; diluted 1:1000) in TBS containing 0.05% Tween 20 (TTBS). The secondary antibodies, goat anti-mouse IgG or goat anti-rabbit IgG, coupled with horseradish peroxidase, were diluted 1:5000 in 1% milk protein or 1% BSA in TTBS, respectively. Specific antibody signals were detected with an enhanced chemiluminescence kit (ECL; Amersham).

Caspase-3 assay. Cells were grown in 6 cm plates to confluence and medium was changed to serum-free media (RPMI 1640 with L-glutamine, 50 μg/ml gentamycin, 10 μg/ml transferrin, and 0.5 mg/ml fetuin) for 3 h. Caspase-3 Assay Kit (Pharmingen) was used for cell extraction and analysis. In brief, cells were washed in PBS and pelleted in a microcentrifuge. Cells were resuspended in 100 μl lysis buffer and kept on ice for 15 min. Extracts were clarified by centrifugation at 4 °C for 10 min in a microcentrifuge. 50 μl extract was incubated with 2.5 μl Caspase-3 Fluorogenic Substrate [*N*-acetyl-Asp-Glu-Val-Asp-AMC (7-amino-4-methylcoumarin)] in 250 μl Hepes buffer, pH 7.5 for 1 hr at 37 °C. Fluorescence was measured in a spectrofluorometer related to cell lysates from untreated cells. Values were normalized against protein concentration determined by the Bradford method.

Cell proliferation. Growth profiles of HC11 cells were obtained using MTT labeling (Boehringer–Mannheim). In brief; 5×10^3 cells were seeded in 96 well plates and MTT was added at different timepoints. The formation of formazan was measured at 550 nm using a Spectramax250 (Molecular Devices Corporation).

Results and discussion

Hyperactive JAK2 constructs

Two JAK2 mutants were constructed (Fig. 1); JAK2D1 containing only the kinase domain (domain 1) of JAK2 and JAKΔD2 deleted of the kinase-like domain (domain 2). Using an in vitro kinase assay we have earlier shown that JAK2D1 protein has higher kinase activity compared to JAKΔD2 [27]. Both of these mutants showed higher activity compared to wild-type JAK2.

DNA binding activity and levels of nuclear STAT5 in HC11 cells expressing JAK2 mutants

To investigate if the kinase activity of the two JAK2 mutants could influence signaling in growing and differentiating HC11 cells, the cells were stably transfected with the two constructs. Activation of STAT5, i.e. DNA-binding activity, was analyzed by EMSA using a β-casein probe which harbors a STAT5 binding site. The clones were grown to confluence in complete medium,

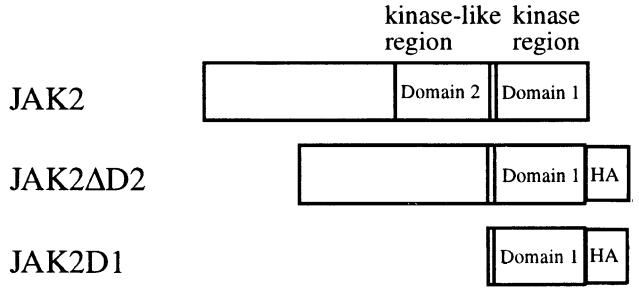

Fig. 1. Schematic presentation of JAK2 constructs. JAK2 wild-type (wt) with the kinase region and the kinase-like region indicated. JAK2ΔD2 is deleted of the kinase-like region (amino acids 551–806). JAK2D1 consists of only the kinase domain (amino acids 855–1129).

i.e. medium containing EGF, insulin and 10% FBS. Thereafter EGF was removed and the serum concentration reduced to 2%, making the cells competent (predifferentiated) to respond to lactogenic hormones [25]. Nuclear extracts were made from HC11 cells at proliferative, confluent, and predifferentiatied states and after treatment of predifferentiated cells with oPRL.

EMSA with nuclear extracts from representative clones of each construct is shown in Figs. 2A and C: HC11-JAK2D1 clones K2 and K4, HC11-JAK2ΔD2 clones Δ1 and Δ6. Low DNA-binding activity was found in nuclear extracts from growing and confluent HC11 cells expressing the hyperactive JAK2D1 (Fig. 2A, lanes 2, 3, 5, and 6). No STAT5 DNA-binding activity was seen in wild-type (wt) non-stimulated HC11 cells (Fig. 2A, lanes 1 and 4). After withdrawal of EGF, no STAT5 DNA-binding activity was detected (Fig. 2A, lanes 7–12). Upon oPRL treatment for 20 min, DNA binding activity was clearly detected in wt and JAK2D1 expressing cells (Fig. 2A, lanes 13–15). The clone K2 possessed higher DNA-binding activity compared with wt and K4 cells. After 90 min of oPRL treatment, STAT5 DNA-binding activity decreased and was not different between the JAK2D1 expressing clones and wt (Fig. 2A, lanes 16–18). In HC11 cells expressing hyperactive JAKΔD2 higher STAT5 DNA binding activity was found after 20 min of oPRL treatment compared with untransfected HC11 cells (Fig. 2C, lanes 13–15). No differences, compared to wt cells, were seen during proliferation, confluence, upon EGF withdrawal, or after 90 min of oPRL treatment.

The nuclear extracts used for EMSA were also analyzed by Western blot using an antibody towards STAT5. HC11 cells expressing hyperactive JAK2D1 were found to have increased nuclear STAT5 protein levels at all states compared with wt HC11 cells (Fig. 2B). HC11 cells expressing hyperactive JAKΔD2 had higher nuclear STAT5 protein levels only upon oPRL treatment compared with wt HC11 cells (Fig. 2D, lanes 13–18).

From the results in Figs. 2A and B it is apparent that no correlation existed between STAT5 protein levels and STAT5 DNA-binding activity in nuclear extracts from JAK2D1 expressing cells. Furthermore, clearly detectable STAT5 DNA-binding activity was only seen in JAK2D1 expressing cells following treatment with oPRL. The prerequisite for nuclear translocation and DNA-binding of STAT proteins is tyrosine phosphorylation [28]. Thus, the nuclear translocated STAT5 in

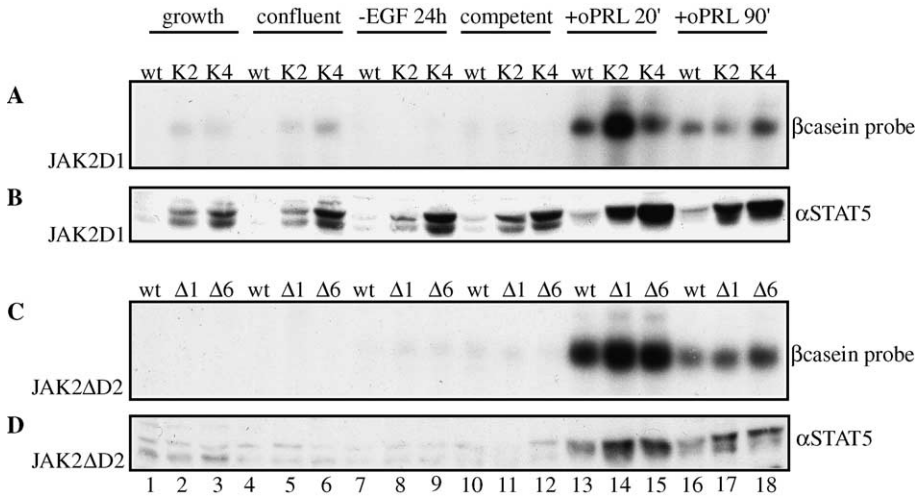

Fig. 2. Nuclear extracts of HC11-JAK2 clones and wild-type HC11 cells assayed for STAT5-DNA binding capacity and STAT5 protein levels. Cells were grown in complete medium until confluence, after which EGF was withdrawn and the serum content was reduced to 2%. Two days later cells were treated with oPRL for 20 or 90 min. Nuclear extracts were prepared during the different culture phases, as indicated in figure. Extracts were analyzed by EMSA using a β-casein probe or separated by SDS–PAGE and analyzed by Western blotting with an antibody directed against STAT5; STAT5A, 94 kDa, and STAT5B, 92 kDa. (A and B) Nuclear extracts of hyperactive JAK2D1 expressing HC11 clones K2 and K4, and wild-type (wt) HC11. (C and D) Nuclear extracts of hyperactive JAK2ΔD2 expressing HC11 clones Δ1 and Δ6, and wt HC11.

JAK2D1 expressing cells must have been rapidly dephosphorylated except when the cells were treated with oPRL. Earlier studies have shown that amino-terminal regions of JAK interact with cytokine receptors [9]. It is thus unlikely that hyperactive JAK2D1 interacted with PRL receptors and responded to PRL stimulation since it lacks domains necessary for receptor interaction. In JAK2D1 expressing cells it is more probable that the expressed kinase domain tyrosine phosphorylated proteins in an unregulated manner. However, Western blot analysis with anti-phosphotyrosine antibody showed that the general level of tyrosine phosphorylation of cellular proteins in JAK2D1 cells was not changed compared to wt cells, suggesting that the overexpressed kinase domain retained the substrate specificity (data not shown). Although the hyperactive JAK2D1 construct induced STAT5 nuclear translocation it seemed to lack the capacity to initiate signal(s) important for STAT5 to retain DNA-binding capacity in the nucleus. It was only when the endogenous PRL receptor was stimulated as clearly detectable STAT5 DNA-binding activity was detected in the JAK2D1 expressing cells. Thus, it is possible that activated PRL receptor/JAK2 complex not only activates STAT5 by tyrosine phosphorylation but also transmits signals to the nuclear compartment that either lower the nuclear capacity to dephosphorylate STAT5 or in an unknown way inhibits STAT5 deactivation. Recently, the nuclear protein tyrosine phosphatase TC-PTP has been shown to interact with and dephosphorylate STAT5 in the nucleus [29]. At present it is unknown if this or a similar nuclear phosphatase taking part in dephosphorylation of STAT5 is regulated by ligand-induced signal(s) or has constitutive activity in vivo.

As described above the only difference found between JAKΔD2 cells and wt cells, was increased PRL responsiveness, i.e., STAT5 activation indicating that overexpressed JAKΔD2 associates with PRLR. This JAK2 mutant contains amino-terminal regions necessary for interaction with PRLR. A likely explanation for the increased nuclear STAT5 protein levels and increased STAT5 DNA-binding activity in these cells is therefore increased kinase activity after PRL treatment. This increased kinase activity could either be due to the presence of more PRLR-JAK2 complexes, increased JAK2 activity after PRL treatment, i.e., higher enzymatic turnover as compared to endogenous JAK2 or sustained JAK2 activity due to impaired down-regulation of JAK2 activity.

Effects of hyperactive JAK2 signaling on growth, differentiation and apoptosis

The involvement of JAK–STAT signaling in regulation of cellular proliferation has earlier been investigated [9, and references therein]. From these studies, which have especially concerned hematopoietic cells, contradictory results regarding JAK–STAT signaling and proliferation have been obtained. We investigated the influence of the mutated JAK2 variants on growth rate of HC11 cells, in the presence or absence of the, for these cells, potent mitogen EGF. No difference in growth rate between mutant expressing and wt cells was found (data not shown). In proliferating HC11 cells expressing hyperactive JAK2D1 a low amount of STAT5 was found to be activated to a DNA-binding form, as described above (Fig. 2A, lanes 2 and 3). The lack of influence on proliferation implies that activated STAT5 does not have a role in regulation of proliferation in HC11 mammary epithelial cells.

One marker of terminal differentiation in mammary epithelial cells is expression of milk proteins. The effect of JAK2 mutants on the expression of the milk protein β-casein in lactogenic hormone treated HC11 cells was investigated. Cells were grown to predifferentiated state and lactogenic hormones were added. Whole cell extracts were prepared after 8 days of hormonal exposure and analyzed by Western blot using an antibody against β-casein (Fig. 3). Despite that both HC11 cells expressing JAK2D1 and JAKΔD2 showed increased activation of STAT5 upon PRL treatment, differences were found when we analyzed β-casein expression. In cells expressing JAKΔD2 β-casein expression was of the same magnitude as in wt cells (Fig. 3, lanes 2–4). Whereas in cells expressing JAK2D1 the β-casein expression was nearly blocked (Fig. 3, lanes 5 and 6). The lack of correlation between STAT5 activation and β-casein expression in HC11 cells expressing hyperactive JAK2D1, suggests that conflicting signals with regard to β-casein expression were triggered by JAK2D1. Earlier studies by Happ et al. [30] demonstrated that HC11 cells transfected with constitutively active Ha-*ras* or v-*raf* oncogenes, components of the mitogenic pathway, assumed transformed properties and were blocked in lactogenic activation of STAT5 and β-casein gene

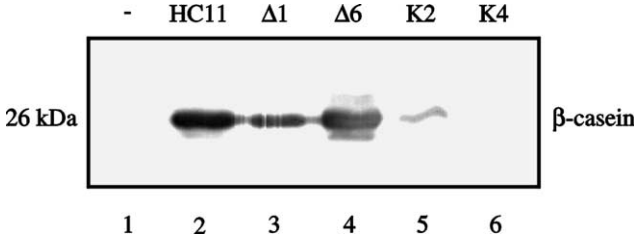

Fig. 3. Lactogenic hormone induced β-casein expression. Cells were grown until confluence in complete medium, then treated with lactogenic hormones in medium without EGF and with 2% FBS medium for 8 days, or left untreated as control (–). Whole cell extracts were resolved by SDS–PAGE and analyzed by Western blotting using an antibody against β-casein. HC11, wildtype HC11 cells; Δ1 and Δ6, HC11 expressing hyperactive JAKΔD2; K2 and K4, HC11 expressing hyperactive JAK2D1.

transcription. This block was not due to transformation in itself as transformation by *int*-2, a member of the fibroblast growth factor gene family, did not interfere with STAT5 activation and β-casein induction. Neither transformation of HC11 by c-*myc* interferred with STAT5 activation, but in this case β-casein induction was hormone independent [30]. Thus, dependent on the oncogene introduced in HC11 cells, different outcomes regarding STAT5 activation and β-casein induction can result.

The ability to undergo apoptosis was analyzed in JAK2 mutant transfected and wt HC11 cells. Cells were grown to confluence and kept in serum-free media for three hours. Cell extracts were prepared and analyzed in a caspase-3 assay. Caspase-3 is a protease activated early in the apoptosis process [31]. As can be seen in Fig. 4 all the clones expressing hyperactive JAK2 mutants showed a decreased ability to undergo apoptosis upon serum withdrawal as compared to wt HC11 cells. In JAK2D1 expressing cells this could be explained by the presence of activated, nuclear STAT5. STAT5 has been suggested to be protective against apoptosis at the onset of involution of mammary gland [32]. In the case of JAK2ΔD2 expressing cells the mutant JAK2 could either activate STAT to a low undetectable level or activate other anti-apoptotic signaling pathway(s). STAT5 has been shown to increase expression of anti-apoptotic proteins, e.g. members of the Bcl-2 family [33]. Thus, one explanation for the effects on apoptosis in hyperactive JAK2 signaling cells could be upregulation of anti-apoptotic proteins. Transgenic mice expressing PRL develop mammary tumors [34]. The intracellular mechanism(s) for this effect is not known. It is likely that mammary epithelial cells in these transgenic mice have increased JAK2/STAT5 signaling. From our in vitro study it can be suggested that JAK2/STAT5 mediated anti-apoptotic effects is one possible mechanism taking part in transformation of mammary epithelial cells.

In summary, in HC11 cells expressing the JAK2 mutant consisting of only the kinase domain, nuclear STAT5 protein level was increased, but no corresponding increase in DNA binding was seen in the absence of PRL stimulation. Furthermore, data from the present study indicate that JAK2 signaling and activation of STAT5 are not involved in regulation of mammary epithelial cell proliferation. HC11 cells expressing either of the two hyperactive JAK2 mutants showed less ability to undergo apoptosis implicating that the mutants have transforming potential. Further studies are needed to understand the mechanism for inhibition of apoptosis in mammary epithelial cells with hyperactive JAK2 signaling.

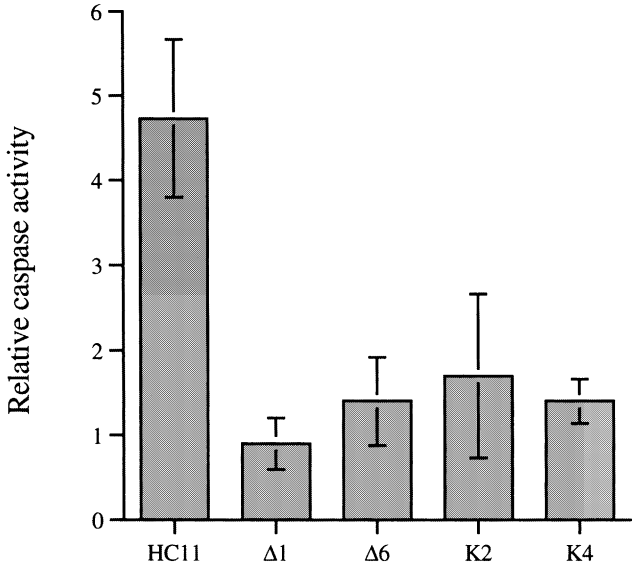

Fig. 4. Apoptosis was analyzed by measuring Caspase-3 activity. Cells were grown to confluence and medium was changed to serum-free medium for three hours. Extracts were prepared and incubated with Caspase-3 fluorogenic substrate. Fluorescence was measured in a spectrofluorometer and compared with untreated cell lysates. Values were normalized against protein concentration determined by the Bradford method.

Acknowledgments

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References

[1] J.N. Ihle, B.A. Witthuhn, F.W. Quelle, K. Yamamoto, O. Silvennoinen, Signaling through the hematopoietic cytokine receptors, Annu. Rev. Immunol. 13 (1995) 369–398.
[2] E. Parganas, D. Wang, D. Stravopodis, D.J. Topham, J.C. Marine, S. Teglund, E.F. Vanin, S. Bodner, O.R. Colamonici, J.M. van Deursen, G. Grosveld, J.N. Ihle, Jak2 is essential for signaling through a variety of cytokine receptors, Cell 93 (1998) 385–395.
[3] S.J. Rodig, M.A. Meraz, J.M. White, P.A. Lampe, J.K. Riley, C.D. Arthur, K.L. King, K.C. Sheehan, L. Yin, D. Pennica, E.M. Johnson Jr., R.D. Schreiber, Disruption of the Jak1 gene demonstrates obligatory and nonredundant roles of the Jaks in cytokine-induced biologic responses, Cell 93 (1998) 373–383.
[4] H. Neubauer, A. Cumano, M. Muller, H. Wu, U. Huffstadt, K. Pfeffer, Jak2 deficiency defines an essential developmental checkpoint in definitive hematopoiesis, Cell 93 (1998) 397–409.
[5] W.J. Leonard, J.J. O'Shea, Jaks and STATs: biological implications. [185 refs], Annu. Rev. Immunol. 16 (1998) 293–322.
[6] J.N. Ihle, Cytokine receptor signalling, Nature 377 (1995) 591–594.
[7] R.M. Weber-Nordt, R. Mertelsmann, J. Finke, The JAK–STAT pathway: signal transduction involved in proliferation, differentiation and transformation [80 refs], Leukemia & Lymphoma 28 (1998) 459–467.
[8] J.M. Shillingford, K. Miyoshi, G.W. Robinson, S.L. Grimm, J.M. Rosen, H. Neubauer, K. Pfeffer, L. Hennighausen, Jak2 is an essential tyrosine kinase involved in pregnancy-mediated devel-

opment of mammary secretory epithelium, Mol. Endocrinol. 16 (2002) 563–570.
[9] S.G. Rane, E.P. Reddy, Janus kinases: components of multiple signaling pathways, Oncogene 19 (2000) 5662–5679.
[10] J.E. Darnell Jr., I.M. Kerr, G.R. Stark, Jak–STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins [97 refs], Science 264 (1994) 1415–1421.
[11] O. Silvennoinen, J.N. Ihle, J. Schlessinger, D.E. Levy, Interferon-induced nuclear signalling by Jak protein tyrosine kinases, Nature 366 (1993) 583–585.
[12] F.W. Quelle, N. Sato, B.A. Witthuhn, R.C. Inhorn, M. Eder, A. Miyajima, J.D. Griffin, J.N. Ihle, JAK2 associates with the beta c chain of the receptor for granulocyte-macrophage colony-stimulating factor, and its activation requires the membrane-proximal region, Mol. Cell. Biol. 14 (1994) 4335–4341.
[13] F.W. Quelle, W. Thierfelder, B.A. Witthuhn, B. Tang, S. Cohen, J.N. Ihle, Phosphorylation and activation of the DNA binding activity of purified Stat1 by the Janus protein-tyrosine kinases and the epidermal growth factor receptor, J. Biol. Chem. 270 (1995) 20775–20780.
[14] R.J. Duhe, W.L. Farrar, Characterization of active and inactive forms of the JAK2 protein–tyrosine kinase produced via the baculovirus expression vector system, J. Biol. Chem. 270 (1995) 23084–23089.
[15] S. Pellegrini, I. Dusanter-Fourt, The structure, regulation and function of the Janus kinases (JAKs) and the signal transducers and activators of transcription (STATs) [228 refs], Eur. J. Biochem. 248 (1997) 615–633.
[16] N.D. Horseman, W. Zhao, E. Montecino-Rodriguez, M. Tanaka, K. Nakashima, S.J. Engle, F. Smith, M. Markoff, K. Dorshkind, Defective mammopiesis, but normal hematopoiesis, in mice with a targeted disruption of the prolactin gene, EMBO J. 16 (1997) 6926–6935.
[17] C.J. Ormandy, A. Camus, J. Barra, D. Damotte, B. Lucas, H. Buteau, M. Edery, N. Brousse, C. Babinet, N. Binart, P.A. Kelly, Null mutation of the prolactin receptor gene produces multiple reproductive defects in the mouse, Genes Dev. 11 (1997) 167–178.
[18] B.K. Vonderhaar, Prolactin—the forgotten hormone of human breast cancer, Pharmacol. Therapeut. 79 (1998) 169–178.
[19] H. Wakao, M. Schmitt-Ney, B. Groner, Mammary gland-specific nuclear factor is present in lactating rodent and bovine mammary tissue and composed of a single polypeptide of 89 kDa, J. Biol. Chem. 267 (1992) 16365–16370.
[20] X. Liu, G.W. Robinson, F. Gouilleux, B. Groner, L. Hennighausen, Cloning and expression of Stat5 and an additional homologue (Stat5b) involved in prolactin signal transduction in mouse mammary tissue, Proc. Natl. Acad. Sci. 92 (1995) 8831–8835.
[21] A.L. Mui, H. Wakao, N. Harada, A.M. O'Farrell, A. Miyajima, Interleukin-3, granulocyte-macrophage colony stimulating factor and interleukin-5 transduce signals through two STAT5 homologs, EMBO J. 14 (1995) 1166–1175.
[22] X. Liu, G.W. Robinson, K.U. Wagner, L. Garrett, A. Wynshaw-Boris, L. Hennighausen, Stat5a is mandatory for adult mammary gland development and lactogenesis, Genes Dev. 11 (1997) 179–186.
[23] S. Teglund, C. McKay, E. Schuetz, J.M. van Deursen, D. Stravopodis, D. Wang, M. Brown, S. Bodner, G. Grosveld, J.N. Ihle, Stat5a and Stat5b proteins have essential and nonessential, or redundant, roles in cytokine responses, Cell 93 (1998) 841–850.
[24] W. Doppler, B. Groner, R.K. Ball, Prolactin and glucocorticoid hormones synergistically induce expression of transfected rat beta-casein gene promoter constructs in a mammary epithelial cell line, Proc. Natl. Acad. Sci. 86 (1989) 104–108.
[25] H. Petersen, L.A. Haldosen, Egf modulates expression of stat5 in mammary epithelial cells, Exp. Cell Res. 243 (1998) 347–358.
[26] J.D. Dignam, R.M. Lebovitz, R.G. Roeder, Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei, Nucleic Acids Res. 11 (1983) 1475–1489.
[27] P. Saharinen, K. Takaluoma, O. Silvennoinen, Regulation of the Jak2 tyrosine kinase by its pseudokinase domain, Mol. Cell Biol. 20 (2000) 3387–3395.
[28] J.E. Darnell Jr., STATs and gene regulation [99 refs], Science 277 (1997) 1630–1635.
[29] N. Aoki, T. Matsuda, A nuclear protein tyrosine phosphatase TC-PTP is a potential negative regulator of the PRL-mediated signaling pathway: dephosphorylation and deactivation of signal transducer and activator of transcription 5a and 5b by TC-PTP in nucleus, Mol Endocrinol 16 (2002) 58–69.
[30] B. Happ, N.E. Hynes, B. Groner, Ha-ras and v-raf oncogenes, but not int-2 and c-myc, interfere with the lactogenic hormone dependent activation of the mammary gland specific transcription factor, Cell Growth Differ. 4 (1993) 9–15.
[31] D.W. Nicholson, A. Ali, N.A. Thornberry, J.P. Vaillancourt, C.K. Ding, M. Gallant, Y. Gareau, P.R. Griffin, M. Labelle, Y.A. Lazebnik, et al., Identification and inhibition of the ICE/CED-3 protease necessary for mammalian apoptosis, Nature 376 (1995) 37–43.
[32] B. Groner, L. Hennighausen, Linear and cooperative signaling: roles for Stat proteins in the regulation of cell survival and apoptosis in the mammary epithelium, Breast Cancer Res. 2 (2000) 149–153.
[33] T. Bowman, R. Garcia, J. Turkson, R. Jove, STATs in oncogenesis, Oncogene 19 (2000) 2474–2488.
[34] H. Wennbo, M. Gebre-Medhin, A. Gritli-Linde, C. Ohlsson, O.G. Isaksson, J. Tornell, Activation of the prolactin receptor but not the growth hormone receptor is important for induction of mammary tumors in transgenic mice, J. Clin. Invest. 100 (1997) 2744–2751.