Prolactin stimulates the JAK2 and focal adhesion kinase pathways in human breast carcinoma T47-D cells

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Treatment of T47-D human breast carcinoma cells with recombinant prolactin (rhPRL) induced a concentration- and time-dependent increase in the phosphotyrosine content of JAK2. rhPRL also stimulated JAK2 tyrosine phosphorylation more weakly in three other breast carcinoma lines, MCF-7, ZR-75-1 and MDA-MB-231. Furthermore it stimulated tyrosine phosphorylation of two isoforms of the transcriptional activator STAT5, STAT5a and STAT5b. Surprisingly, rhPRL treatment of T47-D cells also stimulated tyrosine phosphorylation of focal adhesion kinase (FAK), as determined by immunoprecipitation with anti-phosphotyrosine antibody followed by immunoblotting with a specific FAK antibody. The effect of rhPRL

was rapid and concentration-dependent, being maximal at 5 ng/ml. At rhPRL concentrations above 25 ng/ml, FAK tyrosine phosphorylation declined but remained above control levels at 100 ng/ml. rhPRL also stimulated paxillin tyrosine phosphorylation in T47-D cells with similar concentration- and time-dependence. In a second human breast carcinoma cell line, MCF-7, rhPRL produced very similar effects on FAK and paxillin tyrosine phosphorylation. These findings identify a new protein tyrosine kinase pathway in the action of the lactogenic hormone rhPRL and represent the first report that a hormone acting through a member of the haemopoietin receptor superfamily can regulate the FAK/paxillin pathway.

INTRODUCTION

Prolactin (PRL) is a multifunctional pituitary hormone which regulates a spectrum of physiological processes including mammary-gland development, lactation and immune function and stimulates the proliferation of a variety of cell types including normal and carcinoma mammary epithelial cells [1]. The PRL receptor is a member of the superfamily of cytokine receptors and has the greatest similarity to the receptor for growth hormone [2]. PRL receptors are present in 72 % of breast tumour samples [3] and pituitary-derived PRL is mitogenic in primary breast cancer samples [4]. Recent findings have implicated tyrosine phosphorylation and protein tyrosine kinases in the mechanism of PRL action. Activation of the PRL receptor causes an increase in the tyrosine phosphorylation, activation and receptor association of the non-receptor protein tyrosine kinase JAK2 in rat lymphoma cells, mouse mammary explants and murine lymphoid BAF-3 cells transfected with PRL receptor [5–9]. PRL has also been shown to induce PRL receptor tyrosine phosphorylation and JAK2 association in rabbit mammary glands *in vivo* [10]. PRL also activates the transcription factor MGF-STAT5, which is implicated in induction of milk protein expression [11,12]. The significance of the JAK2/STAT5 pathway for the effects of PRL in human breast carcinoma cells remains unclear, however.

Recent findings suggest that other signal-transduction pathways may be involved in the mediation of PRL-induced biological responsiveness. PRL was reported to induce tyrosine phosphorylation of the adaptor protein SHC in breast carcinoma cells [13] and phosphorylation and activation of Raf-1 kinase in the Nb2 T-cell line [14]. Furthermore it was shown that association of a proline-rich region of the PRL receptor is sufficient for association with and activation of JAK2, as well as activation of STAT1, but not for activation of lactogenic transcription [12].

Several findings indicate that the biological effects of PRL are dependent on interactions between its target cells and the extracellular matrix. Thus it has been established that PRL and basement membrane are both required for milk protein gene expression in mammary epithelial cells and that these two factors act synergistically to induce expression of β -casein [15,16]. In addition, PRL has been reported to cause changes in the adhesive properties and morphology of T47-D breast carcinoma cells [17]. The signalling pathways that mediate the extracellular-matrix-dependent effects of PRL are unknown, although it was recently demonstrated that the ability of STAT5 to bind DNA after activation by PRL is dependent on interactions with basement membrane [18].

FAK is a widely expressed and highly conserved non-receptor protein tyrosine kinase of molecular mass 125 kDa which is localized to focal adhesions and is implicated in the regulation of cytoskeletal organization, cell adhesion and migration [19–21]. Tyrosine phosphorylation of FAK is stimulated by a variety of stimuli including *v-src* transformation [22,23], activation of β_1 -integrins in fibroblasts [24–26], the regulatory peptides bombesin, vasopressin and endothelin [27,28]. Recently, it has been shown that platelet-derived growth factor can also stimulate FAK and paxillin tyrosine phosphorylation in vascular smooth-muscle cells and in Swiss 3T3 cells, indicating that another class of receptor with intrinsic protein tyrosine kinase activity can also regulate this pathway [29,30]. FAK has been shown to associate with other cellular components [20,21], including paxillin, a 68 kDa protein that co-localizes to focal contacts and is a putative substrate for FAK [31–33]. FAK and paxillin tyrosine phosphorylation are associated with the migration of several cell types [30,34,35], and cells from FAK-deficient mouse embryos exhibit a disorganized actin cytoskeleton and reduced cell motility [36]. All these findings suggest that FAK plays a critical role in

Abbreviations used: anti-Tyr(P), antiphosphotyrosine antibody; DMEM, Dulbecco's modified Eagle's medium; FAK, focal adhesion kinase; JAK2, Janus kinase 2; mAb, monoclonal antibody; PRL, prolactin; rhPRL, recombinant human prolactin; STAT5, signal transducer and activator of transcription 5.

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the regulation of cell adhesion, morphology and motility. To date, however, FAK tyrosine phosphorylation through a member of the haemopoietin receptor superfamily has not been reported.

In the present study we investigated the tyrosine phosphorylation events stimulated by rhPRL in human breast carcinoma cells. We report that physiological concentrations of rhPRL stimulate rapid tyrosine phosphorylation not only of JAK2 and STAT5, but also of both FAK and the FAK-associated protein paxillin. This is the first report that a ligand for a member of the cytokine family of receptors can stimulate the FAK protein tyrosine kinase pathway.

Materials and methods

Cell culture

Cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 20% fetal calf serum (Life Technologies, Paisley, Scotland, U.K.). Cells were treated with rhPRL after a wash with PBS and transfer to serum-free medium composed of a 1:1 mixture of DMEM and F12 medium supplemented with Hepes (15 mM, pH 7.2), transferrin (10 µg/ml) and BSA (200 µg/ml) and without Phenol Red [37].

Immunoprecipitation

Cultures of cells (1×10^6) were washed twice with DMEM/F12 (1:1), treated with rhPRL in 10 ml of this medium as indicated and lysed at 4 °C in 1 ml of a solution containing 10 mM Tris/HCl, pH 7.6, 5 mM EDTA, 50 mM NaCl, 30 mM sodium pyrophosphate, 50 mM NaF, 0.1 mM Na_3VO_4 , 1 mM PMSF, 0.5% Nonidet P40 and 1% Triton X-100 (lysis buffer). Lysates were clarified by centrifugation at 15000 *g* for 10 min and transferred to fresh tubes for immunoprecipitation. Immunoprecipitation was routinely performed by incubating lysates with 1 µg/ml antibody as indicated for 2 h at 4 °C. Immunocomplexes were collected by incubating lysates with 1 µg per lysate of anti-mouse IgG for 1 h followed by a 1 h incubation with Protein A-agarose beads. Immunoprecipitates were washed three times with lysis buffer, and proteins were extracted with 2×SDS/PAGE sample buffer before further analysis by SDS/PAGE and Western blotting.

Western blotting

SDS/PAGE of immunoprecipitated proteins was performed as described [30] on gels containing 8% acrylamide. Proteins were then transferred from gels to Immobilon membranes. Membranes were blocked using 5% non-fat dried milk in PBS, pH 7.2, and incubated for 3–5 h in PBS/0.05% Tween 20 containing either anti-Tyr(P) or protein-specific antibodies (1 µg/ml of each) as indicated. Immunoreactive bands were visualized by chemiluminescence using horseradish peroxidase-conjugated anti-mouse IgG and ECL[®] reagent according to the manufacturer's instructions. Autoradiograms obtained from Western blots were scanned using an LKB Ultrosan XL densitometer to quantify tyrosine phosphorylation. Values for tyrosine phosphorylation correspond to the peak areas produced by individual protein bands during scanning and are expressed in terms of fold increase above basal unstimulated levels.

Receptor assay

Iodination of rhPRL was performed using the Iodogen method [38]. Radiolabelled rhPRL was stored at –20 °C in 24 mg/ml glycine. The specific radioactivity of the ^{125}I -rhPRL was 50–200 µCi/µg. Before receptor-binding assays, ^{125}I -rhPRL

was isolated by chromatography on a Superose 12 column (Pharmacia). Binding assays were carried out essentially as described [39]. Cells were seeded in 24-well plates and grown to confluence. After a further preincubation for 18 h in DMEM with 1% fetal calf serum, ^{125}I -rhPRL (50 µl) was added in the absence or presence of increasing concentrations of unlabelled rhPRL (0–500 ng/ml) in assay buffer (PBS containing 1% BSA). Cells were incubated for 4 h at 20 °C, then placed on ice and washed three times with ice-cold assay buffer. Cells were solubilized in 1 M NaOH, and radioactivity was measured in a γ-counter (LKB Wallac 1280-001 Ultrogamma). Specific binding was determined by subtracting radioactivity bound in the presence of 500 ng/ml rhPRL.

Materials

rhPRL was produced as described previously [40] with an additional purification step: rhPRL was loaded on a mono Q HR 10/10 (8 ml) anion-exchange column in 20 mM Tris/HCl, pH 8, and eluted with an NaCl gradient. The concentration of all PRL solutions was checked by protein assay, and biological activity was assessed by the Nb2 bioassay [41]. DMEM/F12 medium containing Hepes and without Phenol Red was supplied by Sigma. Py20 anti-Tyr(P) monoclonal antibody (mAb), and mAbs to FAK, paxillin and STAT5 were from Transduction Laboratories. Antibodies to JAK2 and 4G10 were from UBI. STAT5a and STAT5b were obtained from Santa Cruz Inc. Protein A-agarose and goat anti-mouse IgG were from Oncogene Science. ECL[®] reagents and horseradish peroxidase-conjugated anti-mouse IgG were from Amersham. All other reagents used were of the purest grade available.

RESULTS

Anti-phosphotyrosine immunoblot analysis of anti-Tyr(P) immunoprecipitates prepared from PRL-treated T47-D cells showed that rhPRL stimulated the tyrosine phosphorylation of

Figure 1 PRL stimulation of JAK2 tyrosine phosphorylation in T47-D breast carcinoma cells

Cultures of T47-D cells were washed in serum-free medium and incubated in the same medium with either different concentrations of rhPRL for 30 min (**A**) or with 50 ng/ml rhPRL for different times (**B**). Lysates were prepared, immunoprecipitated with mAb 4G10 and then immunoblotted with anti-JAK2 antibody. The results shown are representative of four independent experiments.

Figure 2 PRL Stimulation of JAK2 tyrosine phosphorylation in human breast carcinoma cell lines

The cell lines indicated were treated with 50 ng/ml rhPRL for 30 min and lysed. Immunoprecipitations were performed with mAb 4G10 and Western blots with anti-JAK2 antibody as described for Figure 1. The results shown are representative of four independent experiments.

Table 1 Scatchard analysis of ^{125}I -rhPRL binding to high-affinity sites in human breast carcinoma cell lines

Receptor numbers and K_d values are means $\pm$ S.E.M. from 12 (T47-D), 5 (MCF-7), 3 (ZR-75-1) and 2 (MDA-MB-231) independent experiments.

Cell line	Receptors per cell	$10^{-9} \times K_d (\text{M}^{-1})$
T47-D	35000 ± 4500	2.5 ± 0.3
MCF-7	17200 ± 1400	1.7 ± 0.2
ZR-75-1	4600 ± 800	1.8 ± 0.5
MDA-MB-231	1300 ± 700	1.7 ± 0.1

multiple bands including several bands in the 110–135 kDa range (results not shown). To investigate tyrosine phosphorylation of JAK2, anti-Tyr(P) immunoprecipitates were prepared from T47-D cells treated with different concentrations of rhPRL for 30 min and then immunoblotted with specific JAK2 mAb. As shown in Figure 1(A), rhPRL increased JAK2 tyrosine phosphorylation in a concentration-dependent manner with a half-maximal effect at 5 ng/ml and a maximum effect at 25–50 ng/ml. The effect of PRL on JAK2 tyrosine phosphorylation was also time-dependent with a detectable increase in tyrosine phosphorylation after 5 min and a maximum effect at 10 min (Figure 1B). The mean increase in JAK2 tyrosine phosphorylation in T47-D cells induced by a 30 min treatment with 50 ng/ml PRL was 2.5-fold above control unstimulated levels ($n = 4$). rhPRL also stimulated JAK2 tyrosine phosphorylation in three other breast carcinoma cell lines, MCF-7, ZR-75-1 and MDA-MB-231 (Figure 2). Semiquantification of the increase in JAK2 phosphotyrosine content in these cell lines showed that PRL-induced JAK2 tyrosine phosphorylation was lower than that induced in T47-D cells with mean increases above basal levels of 1.6, 1.3- and 1.2-fold respectively ($n = 4$). It was consistently observed, however, that these cell lines exhibited higher basal levels of JAK2 tyrosine phosphorylation than T47-D cells (compare Figures 1 and 2). Determination of the number and affinity of PRL-binding sites in these cell lines by Scatchard analysis of ^{125}I -labelled rhPRL binding (Table 1) showed that PRL receptor numbers were highest in T47-D and MCF-7 cells (35000 and 17200 per cell respectively) and significantly lower in ZR-75-1 and MDA-MB-231 cells (4600 and 1300 per cell respectively). Similar to the findings previously reported by Shiu [39], the rhPRL-binding sites were high affinity with K_d values of 1.7×10^9 to $2.5 \times 10^9 \text{ M}^{-1}$. On the basis of these results, our subsequent studies concentrated primarily on T47-D cells.

PRL stimulation of the transcriptional activator STAT5 was

Figure 3 PRL stimulation of STAT5 tyrosine phosphorylation in T47-D cells

Cultures of T47-D cells were washed in serum-free medium and incubated in the same medium with different concentrations of rhPRL for 30 min. Lysates were prepared, immunoprecipitated with mAb Py20 and then immunoblotted with anti-STAT5 antibody. The results shown are representative of three independent experiments. The arrowheads indicate the positions of two STAT5-immunoreactive protein bands of 94 and 92 kDa. The positions of molecular mass markers (kDa) are indicated on the left.

also examined in T47-D cells. Treatment with different concentrations of PRL followed by immunoprecipitation with anti-Tyr(P) and subsequent immunoblot analysis with anti-STAT5 antibody showed that PRL induced an increase in two discrete bands of apparent molecular mass 92 and 94 kDa (Figure 3). The apparent molecular masses of these bands correspond to those previously reported for STAT5-related proteins [42,43]. The effect of rhPRL on STAT5 tyrosine phosphorylation was also time-dependent with a detectable increase at 10 min and a maximum effect at 30 min (results not shown). Several reports have recently demonstrated the existence of different related STAT5 isoforms, including STAT5a and STAT5b [42,43]. We investigated whether the two STAT5-immunoreactive bands shown in Figure 3 correspond to different STAT5-related species. Immunoprecipitation with anti-Tyr(P) followed by Western-blot analysis with a specific antibody to STAT5a or STAT5b showed that PRL induced an increase in the tyrosine phosphorylation of a discrete 92 kDa band (results not shown). A similar experiment in which anti-Tyr(P) immunoprecipitates were immunoblotted with specific antibody to STAT5b also showed a concentration-dependent increase in tyrosine phosphorylation of a 94 kDa species (results not shown). The STAT5b immunoblots also showed a second band of about 92 kDa. This second species is probably the result of cross-reactivity with STAT5a. The tyrosine-phosphorylated STAT5a and STAT5b isoforms migrated with apparent molecular masses closely corresponding to those of the two STAT5-immunoreactive bands shown in Figure 3.

We investigated the possibility that one of the other bands tyrosine-phosphorylated in response to rhPRL in T47-D cells might be the 125 kDa protein FAK. Anti-phosphotyrosine immunoprecipitates were prepared from T47-D cells that had been treated with different concentrations of rhPRL, and the immunoprecipitates were subsequently immunoblotted with a specific anti-FAK antibody. As shown in Figure 4(A), treatment of T47-D cells with 50 ng/ml rhPRL caused a rapid and striking increase in the tyrosine phosphorylation of FAK. In two independent experiments, a detectable increase in FAK phosphotyrosine content occurred as early as 1 min after treatment of T47-D cells with PRL, reached a maximum at 10 min and was sustained for up to 60 min, the longest time examined (Figure 4A). It was noted that PRL-stimulated FAK tyrosine phosphorylation declined somewhat after 20 min. As judged by semiquantitative scanning densitometry, however, FAK tyrosine phosphorylation at 30 min was 75–85% that obtained at

Figure 4 PRL stimulation of FAK tyrosine phosphorylation in T47-D cells

(A) Cells were treated with 50 ng/ml rhPRL for the times indicated, and then lysed and incubated with either mAb Py20 (pY, upper panel) or an anti-FAK mAb (FAK, lower panel). All immunoprecipitates were subsequently immunoblotted with anti-FAK mAb. The autoradiogram shown is representative of two independent experiments. (B) Cells were treated for 30 min with the indicated concentrations of rhPRL, and then lysed and incubated with either mAb Py20 (pY, upper panel) or an anti-FAK mAb (FAK, lower panel). All immunoprecipitates were subsequently immunoblotted with anti-FAK mAb. The autoradiogram shown is representative of four independent experiments. (C) Autoradiograms from four independent experiments were scanned using an LKB Ultrascan XL densitometer to semiquantify FAK tyrosine phosphorylation stimulated by the concentrations of rhPRL indicated. Values shown represent the mean $\pm$ S.E.M. fold increases ($n = 4$) in FAK tyrosine phosphorylation.

10–20 min. The stimulation of FAK tyrosine phosphorylation was also concentration-dependent with a detectable increase occurring at an rhPRL concentration as low as 1 ng/ml; the stimulation was half-maximal at 1 ng/ml and maximal at 5 ng/ml (Figure 4B). In four independent experiments, the mean maximum increase in rhPRL-induced FAK tyrosine phosphorylation was 7-fold above basal unstimulated levels (Figure 4C). At concentrations above 10 ng/ml, rhPRL-induced FAK tyrosine phosphorylation consistently exhibited a marked decline, but remained significantly above basal levels at concen-

Figure 5 PRL stimulation of paxillin tyrosine phosphorylation in T47-D cells and effects of PRL on FAK and paxillin tyrosine phosphorylation in MCF-7 cells

(A) T47-D cells were treated with PRL at the concentrations indicated, lysed and the lysates were incubated with Py20 mAb and then immunoblotted with a specific paxillin mAb. The positions of molecular mass markers (kDa) are indicated on the left. The blot shown is representative of four independent experiments. (B) MCF-7 cells were treated with PRL at the concentrations indicated. Immunoprecipitates of phosphotyrosyl proteins were prepared using mAb Py20 and immunoprecipitates were then immunoblotted with either anti-FAK (upper panel) or anti-paxillin (lower panel) mAbs. The results shown are representative of two independent experiments.

trations up to 100 ng/ml. We verified that anti-FAK immunoprecipitates prepared from cells treated with different concentrations of rhPRL and for different times contained similar amounts of immunoreactive FAK (Figures 4A and 4B).

It was also verified that rhPRL stimulated paxillin tyrosine phosphorylation in T47-D cells. The concentration-dependence for rhPRL-induced paxillin tyrosine phosphorylation was similar to that for FAK with a maximum increase of 3-fold ($n = 4$) at 5 ng/ml and a decline in anti-phosphotyrosine content above 10–25 ng/ml (Figure 5A). rhPRL-stimulated paxillin tyrosine phosphorylation was also rapid, being detectable within 1 min, maximal at 5 min and persisting for up to 60 min after treatment with rhPRL (results not shown).

To evaluate whether PRL stimulation of the FAK/paxillin pathway was confined to T47-D cells, we tested the ability of rhPRL to induce FAK and paxillin tyrosine phosphorylation in MCF-7 cells. As shown in Figure 5(B), rhPRL caused a

concentration-dependent increase in the tyrosine phosphorylation of FAK and paxillin which was half-maximal at 1 ng/ml, reached a maximum at 5 ng/ml and declined slightly at rhPRL concentrations above 25 ng/ml. It was noted that the basal level of FAK and paxillin tyrosine phosphorylation in MCF-7 cells was significantly higher than in T47-D cells.

DISCUSSION

The results presented here show that rhPRL stimulates the tyrosine phosphorylation of JAK2 and STAT5 in a human breast carcinoma cell line, T47-D, expressing moderate numbers of high-affinity binding sites for rhPRL. These results agree with studies of PRL-stimulated tyrosine phosphorylation in other cell types. We also report that rhPRL stimulates the tyrosine phosphorylation of two isoforms of the transcriptional activator STAT5 (STAT5a and STAT5b). In addition, our findings demonstrate that in T47-D cells and another human breast carcinoma cell line, MCF-7, the lactogenic hormone induces a striking increase in the tyrosine phosphorylation of another unrelated non-receptor protein tyrosine kinase, FAK. rhPRL also stimulated the tyrosine phosphorylation of the focal adhesion-associated component and putative FAK substrate, paxillin. Taken together these results show that rhPRL stimulates the FAK pathway in breast carcinoma cells and is the first report that this pathway can be stimulated either through activation of a member of the cytokine receptor superfamily or in breast carcinoma cells.

Our results revealed some differences between the pattern of PRL-induced tyrosine phosphorylation of JAK2 and FAK. Compared with JAK2, PRL-induced FAK tyrosine phosphorylations appeared to be both more rapid and more potent with a detectable increase after 1 min and at 1 ng/ml and maximal increases at 10 min and 10 ng/ml PRL. In contrast, rhPRL-induced JAK2 tyrosine phosphorylation in breast carcinoma cells was only detectable after 5 min and at 10 ng/ml and maximum JAK2 tyrosine phosphorylation required 30 min and 50 ng/ml. Tyrosine phosphorylation of FAK is therefore a very early tyrosine phosphorylation event induced by rhPRL in a cell expressing a moderate level of PRL receptors.

A noteworthy feature of our results is that rhPRL-stimulated tyrosine phosphorylation of FAK exhibited a pronounced bell-shaped dose-response curve, with tyrosine phosphorylation declining at rhPRL concentrations above 10 ng/ml. It is important to emphasize, however, that FAK tyrosine phosphorylation was induced and remained elevated within the range of rhPRL concentrations that mediate the biological effects of PRL. In contrast, however, rhPRL-stimulated JAK2 tyrosine phosphorylation exhibited no decrease at higher PRL concentrations.

The significance of these differences between PRL-induced FAK and JAK2 tyrosine phosphorylation is unclear, although it is plausible that they may be related to a disruptive effect of rhPRL at high concentrations on the actin cytoskeleton, the integrity of which is essential for the maintenance of FAK tyrosine phosphorylation in other cell types [28,29]. It has previously been shown that platelet-derived growth factor inhibits FAK tyrosine phosphorylation at high concentrations in Swiss 3T3 cells and that it concomitantly causes a marked disruption of the actin cytoskeleton and inhibits cell migration [29,30]. We found no readily detectable disruptive effect of rhPRL on actin filament organization in T47-D cells, as indicated by immunofluorescent staining of filamentous actin with FITC-phalloidin (results not shown). It is possible, however, that a

more subtle effect of PRL on the integrity of the actin cytoskeleton may contribute to the bell-shaped concentration-dependence.

Our results also showed that rhPRL stimulates the tyrosine phosphorylation of paxillin in T47-D and MCF-7 cells. These findings are consistent with previous reports that FAK and paxillin are concomitantly tyrosine phosphorylated in other cell types [29,30,32]. The concentration-dependence for paxillin tyrosine phosphorylation was very similar to that for rhPRL-induced FAK tyrosine phosphorylation.

The mechanism through which rhPRL stimulates FAK tyrosine phosphorylation is unclear, although the fact that this effect occurs at physiological concentrations of rhPRL, which also correlate with ¹²⁵I-rhPRL binding to high-affinity sites in breast carcinoma cells, suggests that it is mediated through the PRL receptor. It was recently reported that the Src-family protein tyrosine kinase pp59^{c-fyn} is associated with and activated by the PRL receptor in T-lymphocytes [44]. Given that pp59^{c-fyn} associates with FAK [45,46] and that FAK is tyrosine phosphorylated by pp60^{c-src} in an adhesion-dependent manner [47], it is plausible that stimulation of the FAK pathway through the PRL receptor could be mediated by pp59^{c-fyn}. Regardless of the precise mechanism involved, the results presented here may have implications for our understanding of both the action of PRL in breast cancer cells and the pathogenesis of breast cancer. It is tempting to speculate that PRL stimulation of the FAK pathway may suggest a mechanistic basis for the previously reported effects of PRL on breast carcinoma cell morphology and adhesion [17] as well as the synergism between PRL and the extracellular matrix in the stimulation of milk protein expression [15,16]. Whether or not FAK activation plays a role in increased tumour invasiveness is unknown, but consistent with this notion, recent findings suggest that FAK mRNA and protein are overexpressed in invasive compared with non-invasive tumours, including breast carcinoma [48,49]. These possibilities warrant further investigation. Whatever the precise role of FAK and paxillin tyrosine phosphorylation in PRL-dependent biological responses, the findings presented here provide evidence that FAK is part of a novel signalling pathway in the action of PRL in breast carcinoma cells.

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