Protein Phosphatase 2A, a Negative Regulator of the ERK Signaling Pathway, Is Activated by Tyrosine Phosphorylation of Putative HLA Class II-associated Protein I (PHAPI)/pp32 in Response to the Antiproliferative Lectin, Jacalin*

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Protein phosphatase 2A (PP2A) is a family of mammalian serine/threonine phosphatases that is involved in the control of many cellular functions including those mediated by extracellular signal-regulated kinase (ERK) signaling. While investigating the reversible antiproliferative effect of the dietary lectin, jacalin, which binds the Thomsen-Friedenreich antigen (galactose β1-3 N-acetylgalactosamine α-), we have found that this lectin (30 μg/ml) induces rapid, transient, tyrosine phosphorylation of putative human HLA-DR-associated protein I (PHAPI, also known as the tumor suppressor pp32) in HT29 human colon cancer cells. This is accompanied by the release of PP2A from association with PHAPI, allowing increased phosphatase activity of PP2A (by 42 ± 10% at 10 min) and consequent complete dephosphorylation of the ERK kinase, MEK1/2, by 10 min and of ERK1/2 by 60 min. PHAPI knockdown by RNA interference abolished the effects of jacalin on PP2A activation and MEK inhibition. Thus phosphorylation of PHAPI/pp32 is a critical regulatory step in PP2A activation and ERK signaling.

PP2A was initially thought to act passively and nonspecifically in antagonizing the action of kinases by removal of phosphate groups from their substrates, but it has become clear that it is tightly regulated by mechanisms that include variation in its subunit composition (3), phosphorylation, and/or methylation of its catalytic subunit and signal-regulated targeting to specific subcellular compartments (1–4). PP2A is an important negative regulator of the extracellular signal-regulated kinase (ERK) signaling pathway (5–7) and is involved in the control of many cellular functions including metabolism, transcription, translation, RNA splicing, DNA replication, cell cycle progression, transformation, and apoptosis (1–8).

PHAPI, first purified by affinity with an agarose-conjugated synthetic cytoplasmic region of HLA-DR α chain, is a putative HLA class II-associated cytosolic protein (9), later identified as a phosphoprotein (10). It belongs to a group of proteins containing 20–29-residue leucine-rich repeat motifs. The principal function of these motifs seems to be the provision of a structural framework that allows protein-protein interactions (11). PHAPI is also known as pp32, a potent tumor suppressor (12–15) that is down-regulated in cancer with reciprocal increased expression of pp32r1 and pp32r2, other members of the pp32 gene family (12). Although the physiological role of PHAPI/pp32 is not fully understood, it has been shown to be a potent inhibitor of PP2A in vitro (16, 17) and also promotes apoptosis by caspase-9 activation (18).

In previous studies, we found that dietary lectins that recognize the oncofetal Thomsen-Friedenreich (TF) carbohydrate antigen (galactose β1–3 N-acetylgalactosamine α-) can cause marked effects on the growth of human intestinal epithelial cancer cells (19–23). Since TF antigen can also be a ligand for mammalian lectins including members of the galectin family (24, 25), we have undertaken further investigation of the mechanism underlying these effects. While investigating the mechanism of the reversible antiproliferative effect of the TF-bind- ing lectin from jackfruit (jacalin) (26), we found that this lectin induces rapid, transient, tyrosine phosphorylation of a 30-kDa protein in HT29 human colon cancer cells. We have here identified this protein as PHAPI and shown that its phosphorylation in response to jacalin leads to PP2A activation and subsequent suppression of ERK signaling.

EXPERIMENTAL PROCEDURES

Materials and Cells—Jacalin and agarose-conjugated jacalin were purchased from Vector Laboratories Inc. (Bretton, UK). Fetuin, asialofetuin, and asialo-bovine mucin were obtained from Sigma. Cell culture medium was obtained from Invitrogen. Polyclonal antibodies against phospho-p38 (Thr180/Tyr182), phospho-MEK1/2 (Ser217/Ser221), and MEK1/2 were purchased from Cell Signaling Technology (Beverly, MA).

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The abbreviations used are: PP2A, protein phosphatase 2A; ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinase; MEK, MAPK/ERK kinase; JNK, c-Jun N-terminal kinase; PHAPI, putative HLA class II-associated protein I; siRNA small interfering RNA; TF, antigen, Thomsen-Friedenreich (galactose β1–3 N-acetylgalactosamine α-); APRIL, acidic protein rich in leucines; MS/MS, tandem mass spectrometry.
Anti-phospho-ERK1/2 (Thr183/Tyr185) polyclonal antibody was obtained from Promega (Southampton, UK). Peroxidase-conjugated anti-phospho-tyrosine antibody (PY20) and mouse monoclonal antibody against the PP2A catalytic subunit were purchased from BD Transduction Laboratories. Mouse monoclonal antibody against phospho-c-Jun N-terminal kinase (JNK), goat polyclonal antibody against PHAPI, goat polyclonal antibody against C-subunit of PP2A, and protein A/G plus agarose beads were from Santa Cruz Biotechnology (Santa Cruz, CA). Phosphatase assay kit, rabbit anti-PP2A polyclonal antibody, and mouse monoclonal anti-phosphotyrosine antibody (4G10) were purchased from Upstate Biotechnology (Botoph Claydon, Buckingham, UK). The anti-PHAPI2a/acidic protein rich in leucines (APRIL) rabbit antibodies against MEK1/2, JNK1 (A, sixth panel), or p38 (A, eighth panel). Jacalin induced inhibition of p-MEK1/2 and p-ERK1/2 but not p-JNK and p-p38. On the figure, the circled p indicates phosphorylation. In B, the cells were incubated with or without 30 μg/ml jacalin in the presence or absence of 100 μg/ml asialo-fetuin (ASF) or fetuin for 10 min (B, top two panels) or 60 min (B, bottom two panels) before lysis of the cells and immunoblotted with antibodies against MEK1/2 (B, first panel) or p-ERK1/2 (B, third panel). Each blot was subsequently stripped and reprobed with antibodies against MEK1/2 (B, second panel) or ERK2 (B, fourth panel). The presence of asialo-fetuin or fetuin almost completely abolished jacalin-induced decrease of p-MEK1/2 and p-ERK1/2.

Chemiluminescence immunoblotting detection kit (Pierce) and Fluor-S Imager (Bio-Rad). The optical density of protein bands after immunoblotting was quantified using Quantity One software (Bio-Rad).

**Purification of Jacalin-binding Glycoproteins**—Eighty percent confluent HT29 cells (2 × 10⁶) were washed with phosphate-buffered saline and lysed (in 10 mM Tris, pH 7.6; 5 mM EDTA; 50 mM NaCl; 0.1 mM sodium orthovanadate; 30 mM tetrasodium pyrophosphate; 2 mM phenylmethylsulfonyl fluoride; 1% Triton-100; 50 units/ml aprotinin; and 1 μg/ml leupeptin) on ice for 10 min. The cells were then scraped off, sonicated on salt-ice for 3 × 25 s, and centrifuged for 15 min at 100,000 × g at 4°C. The supernatant was mixed with 150 μl of jacalin-agarose beads (600 μg of jacalin) and rotated for 2 h at 4°C. After six washes with lysis buffer, the jacalin-agarose bound proteins were eluted with SDS-PAGE sample buffer, separated by SDS-PAGE, and transferred to nitrocellulose membranes. The membranes were either stained with Coomassie Blue R250 or probed with peroxidase-conjugated anti-phosphotyrosine antibody.
Identification by SDS-PAGE and Mass Spectrometry of the 30-kDa Protein Phosphorylated in Response to Jacalin—The 30-kDa protein was sliced out from the SDS-polyacrylamide gel and digested with trypsin (10 μg/ml) according to Ref. 27. The resulting peptides were purified using a C18 ZipTip™ (Millipore) and analyzed by mass spectrometry using nanospray delivery of the unfractionated digest to a ThermoFinnigan LCQ Classic mass spectrometer. Ten peptide fragments were analyzed by MS/MS fragmentation analysis, and the results were searched against NCBIR data base using Mascot (www.matrixscience.com).

Preparation of Cell Lysates and Immunoprecipitation of PP2A and PHAPI—The cells with or without prior treatment with 30 μg/ml jactalin were lysed in 10 mM Tris, pH 7.6; 5 mM EDTA; 50 mM NaCl; 0.1 mM sodium orthovanadate; 30 mM tetrasodium pyrophosphate; 2 mM phenylmethylsulfonyl fluoride; 1% Triton-100; 50 units/ml aprotinin; and 1 μg/ml leupeptin) for 30 min at 4 °C. The cell lysates were centrifuged at 16,000 × g for 15 min at 4 °C to remove insoluble material. The supernatants were incubated with primary antibody (against PP2A, PHAPI, or PHAPI2a) for 2 h at 4 °C and were incubated with protein A/G agarose for another hour. The immunoprecipitates were washed four times with lysis buffer and resuspended in SDS-PAGE sample buffer. The samples were heated at 100 °C for 10 min before analysis by electrophoresis and immunoblottting as described above.

PP2A Activity—PP2A activity in PP2A immunoprecipitates was measured using p-nitrophenyl phosphate as substrate (phosphatase assay kit, Upstate Biotechnology). The cells were treated with 30 μg/ml jactalin for various times and then lysed (50 mM HEPES, pH 7.5; 150 mM NaCl; 1 mM EDTA; 10% glycerol; 1.5 mM magnesium chloride; 1% Triton X-100; 1 mM phenylmethylsulfonyl fluoride; 1 μg/ml leupeptin; and 50 units/ml aprotinin). The lysates were centrifuged at 16,000 × g for 15 min at 4 °C, and the supernatants were incubated with 2.5 μg of anti-PP2A antibody for 2 h and were incubated with protein A/G agarose for a further hour at 4 °C. The immunoprecipitates were washed twice with lysis buffer, washed once with 50 mM Tris buffer (50 mM Tris, pH 7.0; 0.1 mM CaCl2; 2.5 mM NiCl2, and 1 mg/ml p-nitrophenyl phosphate), and incubated at 37 °C for 30 min. The reaction was stopped by the addition of 13% K2HPO4, and the absorbance was read at 405 nm. An aliquot of the cells was pretreated with 30 μM of the specific tyrosine kinase inhibitor genistein for 30 min at 37 °C before the addition of jactalin and subsequent lysis of the cells and immunoprecipitation.

**PHAPI Knockdown by siRNA—Twenty-one-nucleotide small interfering RNA duplexes (siRNA) (first strand, 5′-GGAGCCGUUCUGAU-GUGAATT-3′, second strand, 5′-UUCACAUAGAGCGCCUCCTT-3′) were chemically synthesized by Eurogentec (Seraing, Belgium). Subconfluent HT29 cells were seeded in 24-well plates (2 × 103 cells/well) in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum for 24 h at 37 °C. siRNA duplex was introduced to the cells in the presence of 8 μl/ml TransIT-TKO transfection reagent (Cambridge Bioscience). The cells were cultured at 37 °C for 1–3 days before lysis in SDS-sample buffer. The expressions of PHAPI, PP2A, and MEK after siRNA treatment were assessed by immunoblotting.

**RESULTS**

Tyrosine Phosphorylation of HT29 Cellular Proteins in Response to Jacalin—Jacalin, at a concentration (30 μg/ml) shown previously to cause maximal growth inhibition (26), induced rapid tyrosine phosphorylation of a 30-kDa protein (protein 30) in HT29 human colon cancer cells (Fig. 1A). Two minutes after the addition of jacalin, a transient 390% increase of protein 30 tyrosine phosphorylation was observed that gradually returned to its baseline level over 120 min (Fig. 1, A and B). This effect was not seen in the presence of the TF-expressing glycoprotein asialo-fetuin (data not shown).

**Jacalin Inhibits MAPK Activation—**Jacalin induced transient inhibition of phosphorylation of ERK1/2 and of its upstream kinase regulator MEK1/2 (Fig. 2), in keeping with its antiproliferative effect. MEK1/2 phosphorylation started to decrease by 2 min, becoming undetectable by 5 min but returning to its baseline level after 120 min (Fig. 2A, top panel). Jacalin also induced inhibition of ERK1/2 activity, but this only became significant after 20 min (Fig. 2A, third panel). By 40 min, ERK1/2 activity was reduced by 77%, and by 60 min, its activ-
FIG. 4. Confirmation of protein 30 as PHAPI by jacalin (JAC)-agarose precipitation, immunoprecipitation (IP), and immunoblotting (IB). A, 50 μg of HT29 protein extracts were mixed with or without 25 μl of agarose-jacalin beads (100 μg of jacalin) for 2 h at 4 °C. Agarose-jacalin beads were collected from the protein extracts by centrifugation at 3000 × g for 5 min and washed four times with protein lysis buffer. Cell extracts before (A, lane 1) or after (A, lane 2) agarose-jacalin precipitation and agarose-jacalin precipitates (A, lane 3) were immunoblotted with anti-PHAPI (A, left panel) or anti-PHAPI2a (A, right panel). Each blot was stripped and rebotted with anti-γ-tubulin antibody (A, bottom panels). Agarose-jacalin affinity precipitation removed almost all PHAPI and PHAPI2a from the protein extracts, and γ-tubulin in the extracts suffered only about 22% loss during the precipitation and washing process. In B and C, HT29 cells were incubated with (B and C, first lanes) or without (B and C, second lanes) 30 μg/ml jacalin for 10 min at 37 °C. The cells were lysed, and 250 μg of protein were immunoprecipitated with anti-PHAPI2a (B, first two lanes), anti-PHAPI antibody (C, first two lanes), rabbit IgG (B, third lane), or goat IgG (C, third lane). The resulting immunoprecipitates were immunoblotted with anti-phosphotyrosine antibody (B and C, first panel). Each blot was stripped and reprobed with anti-PHAPI2a (B, second lane) or PHAPI (C, second panel). Jacalin treatment of the cells results in an increase of tyrosine phosphorylation of PHAPI but not of PHAPI2a.

FIG. 5. PHAPI tyrosine phosphorylation causes dissociation of PHAPI from PP2A. A, HT29 cells were treated with 30 μg/ml jacalin for various times and lysed. 500 μg of protein lysate were immunoprecipitated (IP) with 20 μl of anti-PHAPI antibody followed by immunoblotting (IB) with antibodies against phosphotyrosine (A, top panel). The same blot was subsequently stripped twice and probed with anti-PP2A antibody (A, middle panel) or anti-PHAPI antibody (A, lower panel). The optical densities of the relevant protein bands from four experiments were quantified and expressed as the percentage of initial PHAPI (B) and PHAPI-PP2A association (the OD of the PP2A band at each time point was expressed as a percentage of the OD of the PHAPI band at the same time point, mean ± S.D.) (C). PP2A was identified in PHAPI immunoprecipitates, implying association between the two molecules (A, middle panel). The increased PHAPI tyrosine phosphorylation induced by jacalin was accompanied by the decreased association of PP2A with PHAPI. In reciprocal experiments, cells treated with or without 30 μg/ml jacalin were lysed and immunoprecipitated with anti-PP2A antibody (or mouse IgG) followed by immunoblotting using anti-PHAPI antibody (D, upper panel). The blot was stripped and rebotted with anti-PP2A antibody (D, lower panel). Jacalin again caused transient (5–60 min) dissociation of PHAPI from PP2A. *, p < 0.05 (two-tailed t test). On the figure, the circled p indicates phosphorylation. p-PHAPI, phosphorylated PHAPI; PP2A, PHAPI.
without 30 g/ml jacalin for another 10 min at 37°C (A). The cells were lysed, and 40 µg/lane (A, top panel) or 60 µg/lane of protein lysate (A, bottom panel) were immunoblotted (IB) using antibodies against phosphotyrosine (A, top panel) or phospho-MEK1/2 (indicated by p-MEK) (A, third panel). Each blot was stripped and reprobed with antibody against PHAPI (A, second panel) or MEK1/2 (A, bottom panel). Pretreatment of the cells with genistein (Gen) or asialo-fetuin (ASF) prevented jacalin-induced PHAPI tyrosine phosphorylation and down-regulation of MEK1/2. On the figure, the circled p indicates phosphorylation. p-PHAPI, phospho-PHAPI. In B, the cells pretreated with or without 30 µM genistein for 30 min were incubated with or without 30 µg/ml jacalin for 10 min. The cells were lysed, and 500 µg/lane (B) were immunoprecipitated (IP) with 10 µl of anti-PHAPI antibody followed by immunoblotting using anti-PHAPI antibody (B, top panel). The blot was subsequently stripped and reprobed with anti-PP2A (B, bottom panel). In C, HT29 cells, treated similarly as in B, were immunoprecipitated with anti-PP2A antibody. PP2A activity in the immunoprecipitates was determined by using p-nitrophenyl phosphate as substrate and expressed as percentage compared with untreated cells. The data represent two separate experiments each performed in triplicate and expressed as a percentage of initial PP2A activity (mean ± S.D.). Pretreatment of the cells with genistein prevented the jacalin-induced increase of PP2A activity. *, p < 0.05 (two-tailed t test).

**Fig. 7.** Jacalin-induced PHAPI tyrosine phosphorylation, dissociation of PHAPI from PP2A, and induction of PP2A activity are all inhibited by the tyrosine kinase inhibitor genistein and by the TF-expressing glycoprotein asialo-fetuin. HT29 cells were incubated with or without 30 µg/ml asialo-fetuin for another 10 min at 37°C (A). The cells were lysed, and 40 µg/lane (A, top panel) or 60 µg/lane of protein lysate (A, bottom panel) were immunoblotted (IB) using antibodies against phosphotyrosine (A, top panel) or phospho-MEK1/2 (indicated by p-MEK) (A, third panel). Each blot was stripped and reprobed with antibody against PHAPI (A, second panel) or MEK1/2 (A, bottom panel). Pretreatment of the cells with genistein (Gen) or asialo-fetuin (ASF) prevented jacalin-induced PHAPI tyrosine phosphorylation and down-regulation of MEK1/2. On the figure, the circled p indicates phosphorylation. p-PHAPI, phospho-PHAPI. In B, the cells pretreated with or without 30 µM genistein for 30 min were incubated with or without 30 µg/ml jacalin for 10 min. The cells were lysed, and 500 µg/lane (B) were immunoprecipitated (IP) with 10 µl of anti-PHAPI antibody followed by immunoblotting using anti-PHAPI antibody (B, top panel). The blot was subsequently stripped and reprobed with anti-PP2A (B, bottom panel). In C, HT29 cells, treated similarly as in B, were immunoprecipitated with anti-PP2A antibody. PP2A activity in the immunoprecipitates was determined by using p-nitrophenyl phosphate as substrate and expressed as percentage compared with untreated cells. The data represent two separate experiments each performed in triplicate and expressed as a percentage of initial PP2A activity (mean ± S.D.). Pretreatment of the cells with genistein prevented the jacalin-induced increase of PP2A activity. *, p < 0.05 (two-tailed t test).
rosine antibodies confirmed that the protein 30 band contains both PHAPI and PHAPI2a but again showed that increased tyrosine phosphorylation induced by jacalin occurred only on PHAPI and not on PHAPI2a (data not shown).

PHAPI/pp32 is a cytoplasmic/nuclear protein, but experiments using agarose-immobilized, thus non-internalized, jacalin showed that this retained the antiproliferative activity of jacalin (data not shown). This implies that some intermediate interaction between jacalin and a cell surface glycoprotein occurs. Moreover, immunoprecipitated PHAPI/pp32 showed no binding by jacalin when assessed by lectin blotting (data not shown), suggesting that PHAPI interacts with a cell surface jacalin ligand.

PHAPI Tyrosine Phosphorylation and PHAPI-PP2A Interaction—PHAPI/pp32 has been shown to bind, in cell-free systems, to the catalytic C subunit of protein phosphatase 2A (PP2A) and acts as a non-competitive, specific inhibitor of PP2A but not of other phosphatases including PP1, PP2B, PP2C, or pyruvate dehydrogenase phosphatase (16, 17). The nature and regulation of this interaction between PHAPI/pp32 and PP2A has previously been unknown. This, together with our finding that PHAPI tyrosine phosphorylation induced by jacalin is associated with down-regulation of MEK and ERK activity, suggested to us that PHAPI might form a functional complex with PP2A and that tyrosine phosphorylation of PHAPI could regulate association/dissociation of this complex and thus play an important regulatory role in PP2A activation and ERK signaling.

Immunoprecipitation of PHAPI followed by PP2A immunoblotting confirmed an association of PP2A with PHAPI in HT29 cells (Fig. 5, A–C). Within 5 min of application, jacalin (30 μg/ml) induced a marked increase (235%) of tyrosine phosphorylation of PHAPI (Fig. 5, A and B) that was associated with transient dissociation (73%) of PHAPI from PP2A (Fig. 5, A and C). The transient dissociation of PHAPI from PP2A lasted about 60 min and was closely correlated with the increased tyrosine phosphorylation of PHAPI (Fig. 5A). In reciprocal experiments, immunoprecipitation of the PP2A catalytic C subunit followed by PHAPI immunoblotting again showed an association of PP2A with PHAPI, and a similar decrease in the association of PP2A with PHAPI occurred between 5 and 60 min after the introduction of jacalin (Fig. 5D).

To eliminate the possibility that binding to the cellular PP2A by the anti-PP2A antibody was affected by potential posttranslational modification of PP2A such as tyrosine phosphorylation, the experiments were performed with two anti-PP2A antibodies. One was generated by using a synthetic peptide mapping at the C terminus of the PP2A catalytic subunit (Santa Cruz Biotechnology), and the other was generated using the C-terminal half (sequence 153–309) of the PP2A catalytic subunit (BD Biosciences), and similar results were obtained with both antibodies (data not shown). Furthermore, no difference in tyrosine phosphorylation of PP2A immunoprecipitates before and after jacalin (30 μg/ml) treatment was detected by immunoblotting using anti-phosphotyrosine antibody (data not shown).

PHAPI Tyrosine Phosphorylation and PP2A Activation—The transient increase of PHAPI phosphorylation and the dissociation of PHAPI from PP2A induced by jacalin were synchronous with a transient increase of PP2A enzyme activity in these cells (Fig. 6). Two minutes after the addition of jacalin (30 μg/ml), a 36% increase of PP2A activity was observed. Maximal increase (42%) of PP2A activity appeared at 10 min and decreased thereafter, returning to its baseline level by 2 h.

Jacalin-induced increase (245%) of PHAPI tyrosine phosphorylation at 10 min was largely prevented by pretreatment of

![Image](http://www.jbc.org/Downloaded从/333x263_to_547x737)
The cells with 20 μm genistein, a specific tyrosine kinase inhibitor (29), and also by the presence of 100 μg/ml asialo-fetuin (Fig. 7A). Genistein pretreatment was accompanied by a complete abrogation of the effects of jacalin on the decreased association (71%) of PHAPI with PP2A (Fig. 7B) and on the increased activation (34%) of PP2A (Fig. 7C). The 95% decrease of MEK activity in response to jacalin was also abrogated by genistein and by asialo-fetuin (Fig. 7A).

To further investigate the relationship between PHAPI and PP2A, PHAPI expression was suppressed by RNA interference. A synthetic siRNA duplex (si172) designed to target the 172–192 region of PHAPI mRNA was introduced to HT29 cells. PHAPI expression was suppressed 64% by 100 nM si172 by 2 days (Fig. 8A). The introduction of si172 to the cells had no effect on the expression of PP2A, MEK1/2, or tubulin (Fig. 8A). Cell morphology and viability were not significantly affected by si172 within this period (data not shown). This reduction of PHAPI expression by si172 resulted in complete abolation of the effects of jacalin on PP2A activation (Fig. 8B) and subsequent MEK1/2 inhibition (Fig. 8C).

DISCUSSION

These studies show that treatment of HT29 colon cancer cells with the antiproliferative lectin, jacalin, induces tyrosine phosphorylation of PHAPI/pp32, which results in dissociation of PP2A from PHAPI and activation of PP2A followed by inhibition of MEK1/2 and ERK1/2 activity. As PHAPI binds specifically to the catalytic C subunit of PP2A (16, 17), which is present in all of the various PP2A complexes, it seems likely that PHAPI phosphorylation represents a general mechanism for the regulation of PP2A activity and thus for control of the wide range of cellular functions that are regulated by PP2A.

Dephosphorylation of MEK1/2 in response to jacalin occurs much earlier and more completely than that of ERK1/2. The time course suggests that PHAPI phosphorylation represents a general mechanism for dephosphorylation of MEK1/2 and possibly of other kinases in the ERK cascade, than by a direct action on ERK1/2 itself.

Further studies will need to elucidate the nature of the cell surface receptor for jacalin that is responsible for initiating phosphorylation of PHAPI/pp32. The carbohydrate specificity of jacalin raises the possibility that this receptor might represent a natural ligand for one or more members of the naturally occurring galectin (galactose-binding lectin) family (30, 31) and that such a lectin might have an important functional effect by a similar mechanism. It also suggests a functional role for the alteration in cell surface glycosylation, including increased expression of Thomsen-Friedenreich antigen, that is commonly found in malignant and premalignant epithelia (32).

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