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# Shift syndecan-2 from RACK1 to caveolin-2 upon transformation with oncogenic *ras* ☆

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**Abstract**

Syndecan-2 was found to detach from RACK1 and associate with caveolin-2 and Ras in cells transformed with oncogenic *ras*. Most of syndecan-2 from transformed cells was revealed with negligible phosphorylations at tyrosine residues. We experimented with HeLa cells transfected with plasmids encoding syndecan-2 and its mutants (syndecan-$2^{Y180F}$, syndecan-$2^{Y192F}$, and syndecan-$2^{Y180,192F}$) to provide evidences that $PY^{180}$ of syndecan-2 is a binding site for RACK1 and is deprived in cells transfected with oncogenic *ras*. However, in HeLa cells transfected with syndecan-$2^{Y180F}$, RACK1 was found to sustain its reactions with syndecan-2 independent of phosphorylation. The finding of syndecan-2 reactive with caveolin-2/Ras suggests the molecular complex most likely to obstruct RACK1 for functional attachment at syndecan-2, as revealed in cells transfected with oncogenic *ras*. We provided evidences to reinforce the view that molecular rearrangements upon transformation are specific and interesting.


*Keywords:* Syndecan-2; Caveolin-2; RACK1; Ras; Src; Tyrosine phosphorylation

Thirty percent of all human cancers express oncogenically activated *ras*, and its occurrence is common in patients with pancreatic cancer (90%), colorectal cancer (50%), and lung cancer (20%) [1]. Ras proteins are plasma membrane-bound GTP-binding proteins that play a key role in mitotic signal transduction [2]. Mutations that activate Ras in the GTP-locked form constitutively result in uncontrolled cell growth and a common contributor to malignant transformation [3]. The Ras protein is synthesized as a cytosolic precursor and is localized to the inner plasma membrane only after it undergoes a series of post-translational modifications that are triggered by prenylation [2].

Mammalian cells express three *ras* genes that encode four Ras proteins (H-, N-, $K_A$-, and $K_B$-Ras), and the most prevalent activated Ras in human cancers is of the K-type [1]. All mammalian Ras proteins are prenylated by protein farnesyltransferase [4], except $K_B$-Ras, which is additionally acylated by protein geranylgeranyltransferase I [5,6]. Similarly prenylated mechanism was evidenced in S-Ras of shrimp *Penaeus japonicus* which shares 85% homology with mammalian $K_B$-Ras protein, with identity in the guanine nucleotide binding domains, but differences in the lysine-rich region, which is replaced with arginine clusters in S-Ras [7].

Syndecans are located between extracellular matrix and intracellular cytoskeleton [8]. The amount of cell surface syndecan is controlled by reduced expression or shedding in accord with morphological changes, as induced by transformation with oncogenic *ras* [9]. The syndecans are a four-member family of transmembrane cell surface heparan sulfate proteoglycans that are characterized by highly conserved transmembrane and cytoplasmic domains [10], and molecule distinctive extracellular domain to carry out similar but non-identical functions [10,11]. Four conserved

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tyrosine residues ($Y^{170}$, $Y^{180}$, $Y^{192}$, and $Y^{201}$) that are 100% conserved among four vertebrate syndecans in mammals [10,11] that are modified by Src [12–15]. This phosphorylation event may result in a shift of syndecans from non-raft to caveolae [16].

Caveolae are composed of caveolins, a family of 20–24 kDa integral membrane proteins [17]. The N-terminal and C-terminal domains of caveolin face the cytoplasm [18,19], allowing them to interact freely as a ''molecular switch'' to inactivate and sequester GTP binding proteins such as H-Ras, as well as Src. Four novel caveolin-related proteins, caveolin-1α, caveolin-1β, caveolin-2, and caveolin-3, have been identified in mammals [20–22], caveolin-1α and caveolin-1β being splice variants of the same gene.

Cellular signal transduction cascades are regulated through precise compartmentalization of signaling proteins. Compartmentalization of signaling proteins such as kinase and phosphatases is achieved by their interaction with scaffolding proteins [23,24], such as receptors for activated C-kinase (RACKs). Two RACKs have been identified; RACK1 is a homologue of the guanine nucleotide-binding protein (G protein) β subunit [25,26], and RACK2 is the coatomer protein β′-COP [27].

The present study was designed to test whether syndecan-2 functions as a specific site to rearrange molecules for oncogenic *ras* signaling. Therefore, we experimented with mammalian BALB/3T3 cells transfected with plasmids containing the S-*ras*($Q_{61}K$) to determine whether syndecan-2 associated directly with Ras. Because protein phosphorylation plays a critical role in the generation of intracellular signals in response to external stimuli, we investigated whether syndecan-2 becomes phosphorylated or not upon transformation with oncogenic *ras*, and whether this event interrupts the reaction of syndecan-2 with scaffolding proteins, such as RACK1. HeLa cells transfected with plasmids containing the syndecan-2 and its mutants (syndecan-$2^{Y180F}$, syndecan-$2^{Y192F}$, and syndecan-$2^{Y180,192F}$) were applied to assess the reaction between syndecan-2 and RACK1. Finally, we asked whether the reaction of syndecan-2 with RACK1 is dependent on external cytokines. We provided evidences to reinforce the view that molecular rearrangement for aberrant signaling upon transformation is specific and interesting as that for cytokines.

## Materials and methods

*Materials.* All reagents used were of the highest grade available commercially. [γ-$^{32}$P]Adenosine5′-triphosphate ([γ-$^{32}$P]ATP) was from Amersham (Buckinghamshire, UK). ATP and granylocyte-macrophage colony-stimulating factor (GM-CSF) were from Roche Molecular Biochemicals (Penzberg, Germany). Protein A and Protein G magnetic microbeads and μMAS (magnetic Sorting) columns for small scale (approximately 100 μl) molecular biology applications were purchased from Miltenyi Biotec (Bergisch Gladbach, Germany). Reagents for polyacrylamide gel electrophoresis were obtained as described previously [28].

*Cells.* HeLa and BALB/3T3 cells were grown at 37 °C in minimal Eagle's medium (MEM; Life Technologies; Gaithersburg, MD) supplemented with 10% (v/v) fetal bovine serum (Life Technologies; Gaithersburg, MD), penicillin (100 U/ml), and streptomycin (100 μg/ml) separately in an atmosphere of 5% $CO_2$, 95% air.

*Mutation and construction of syndecan-2 plasmids.* Mutant syndecan-$2^{Y180F}$, syndecan-$2^{Y192F}$, and syndecan-$2^{Y180,192F}$ were produced by the overlap extension PCR method in human syndecan-2 cDNA [29,30] and were constructed with pcDNA6.2/GFP-DEST vectors (Invitrogen, Carlsbad, CA) for expression in mammalian cells. The data were reconfirmed with Sequenase Kit (US Biochemical Co., Cleveland, OH).

*Transfections.* BALB/3T3 cells were transfected with shrimp mutant *ras* plasmids, pcDNA3.1-[S-*ras*($Q_{61}K$)], using Effective Transfection Reagent (Qiagen, Hilden, Germany) as specified previously [31]. HeLa cells were transfected with plasmids pcDNA6.2/GFP-DEST containing syndecan-$2^{Y180F}$, syndecan-$2^{Y192F}$ or syndecan-$2^{Y180,192F}$ using lipofectamine 2000 transfection Reagent (Invitrogen, Carlsbad, CA).

*Production of human syndecan-2-encoded and human syndecan-$2^{Y180F}$-encoded, fusion proteins in bacteria.* The PCR products of human syndecan-2 and human syndecan-$2^{Y180F}$ were further cloned into expression vector pET102 (Invitrogen) and expressed in *Escherichia coli* separately. Each protein mixture was loaded onto the $Ni^{2+}$ charged HiTrap chelating affinity column. The column was equilibrated and washed with 10 × column volume of 20 mM sodium phosphate buffer and 10 mM imidazole. Syndecan-2 protein was eluted with 150 mM imidazole. The eluant was dialyzed overnight employing 10 L of 25 mM Tris, pH 7.5, containing 10 mM NaCl. The protein solution was separately concentrated by ultrafiltration in centricon (Amicon, Beverly, MA) and stored in aliquots at −80 °C.

*Preparation of membrane lysates from cells.* Cells were harvested and homogenized in 10 ml of buffer [25 mM MES/pH 6.5, 1 μg/ml leupeptin, 1 μg/ml aprotinin, and 15 mM 2-mercaptoethanol, plus 2.5% (w/v) phenylmethane-sulfonyl fluoride dissolved in propanol, at a dilution of 1:1000] with a Polytron tissue grinder (three 10-s bursts; Kinematica GmbH, Brinkmann Instruments, Westbury, NY). The homogenate was centrifuged at 100,000*g* for 1 h. The recovered pellet was suspended in 25 mM MES, pH 6.5, and incubated with 2% (w/v) Triton X-100 at 37 °C for 10 min. After centrifugation at 10,000*g* for 30 min, the supernatant was subjected to immunoprecipitation.

*In vitro phosphorylation.* The purified *E. coli* expressed syndecan-2 protein preparation (1 μg) was incubated with one unit of c-Src kinase (Upstate) in 100 μl kinase reaction buffer (50 mM PIPES, pH 6.9, 5 mM $MnCl_2$, 10 mM $MgCl_2$, and 2 mM dithiothreitol), and the reaction was initiated by addition of 10 μCi of [γ-$^{32}$P]ATP. After 10 min at 37 °C, the reaction was stopped by addition of 2× Tricine–SDS–PAGE sample buffer and boiling for 2 min.

*Polyacrylamide gel electrophoresis.* Tricine–sodium dodecyl sulfate–polyacrylamide gel electrophoresis (Tricine–SDS–PAGE) was conducted on slab gels that contained 12% (w/v) acrylamide and 0.61% (w/v) *N,N′*-methylenebisacrylamide [32]. Samples were reduced and alkylated [33] before application to the gels. Gels were silver-stained by the method of Merril et al. [34].

Phosphoproteins were separated by Tricine–SDS–PAGE (12% acrylamide) and detected by exposure of dried gel to Kodak BioMax-MS film at −70 °C under an intensifying screen. Radiolabeled bands were quantified with a scanning PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

*Western immunoblotting.* Proteins that had been separated by Tricine–SDS–PAGE were transferred electrophoretically to nitrocellulose membranes (Bio-Rad, Richmond, CA) and probed by Western immunoblotting with antibodies as specified recently [29,30].

## Results

### *Syndecan-2 associates with Ras and caveolin-2 in cells transformed with oncogenic ras*

Membrane proteins were effectively released in soluble form by treatment with warm Triton X-100 from membranes

of BALB/3T3 cells transfected with plasmid pcDNA3.1 or pcDNA3.1-[S-*ras*($Q_{61}$K)] [29,30]. The membrane lysates of cells transformed with oncogenic *ras* or not were immunoprecipitated with antibodies against syndecan-2 and counterstained on a Western blot with antibodies against caveolin-1, and caveolin-2, and Ras (Fig. 1). The syndecan-2 of transformed cells was competent at recruiting caveolin-2, but not caveolin-1 (Fig. 1d), as compared with cells without transformation (Fig. 1b). Residual amount of Ras was found with syndecan-2 in transformed cells (Fig. 1d), but not in cells without transformation (Fig. 1b).

*Syndecan-2 exhibits less phosphorylation at tyrosine residues in cells transformed with oncogenic ras*

Protein phosphorylation plays a critical role in the generation of intracellular signals in response to external stimuli, such as oncogenic Ras. The membrane lysates of BALB/3T3 cells transfected with either plasmids pcDNA3.1 or pcDNA3.1-[S-*ras*($Q_{61}$K)] were immunoprecipitated with monoclonal antibodies against phosphotyrosine (PY) or phosphoserine (PS) and counterstained on a Western blot with antibodies against syndecan-2 (Fig. 2A) and caveolin-2 (Fig. 2B). The syndecan-2 of cells transformed with oncogenic *ras* or not was competent at reacting with anti-PS (Fig. 2A). In contrast, little syndecan-2 was reactive with anti-PY in transformed cells (Fig. 2A). In other words, upon transformation with oncogenic *ras*, the phosphorylation at tyrosine residues of syndecan-2 was found to be depressed (Fig. 2A). Of interests, caveolin-2, a partner of syndecan-2 in transformed cells (Fig. 1), was heavily phosphorylated at tyrosine residues (nearly 3-folded; Fig. 2B) as well as at serine residues (Fig. 2B). Data suggest that a tyrosine kinase (or tyrosine/serine kinase) might shift its substrate from syndecan-2 to caveolin-2 in cells transfected with oncogenic *ras*. Such a substrate selection difference is most likely mediated by the scaffold protein RACK1, a scaffold protein to

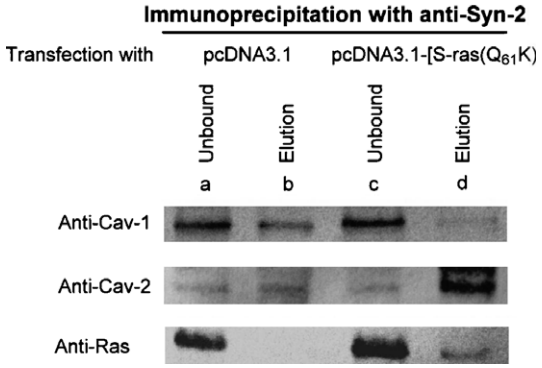


Fig. 1. Caveolin-2 and Ras are selectively recruited with syndecan-2 in cells transfected with oncogenic *ras*. Cells ($10^6$) transfected with plasmids of pcDNA3.1-[S-*ras*($Q_{61}$K)] were homogenized and membrane lysates were incubated with rabbit polyclonal antibody against syndecan-2 (1 μg) at 37 °C for 30 min. The immunoprecipitate recovered in the elution buffer (100 μl) containing 25 mM Hepes, pH 7.5, and 1 M NaCl (d) and the previous unbound fraction (c) were subjected to electrophoresis on a Tricine–SDS–PAGE (12%), and Western blotting with mouse monoclonal antibody specific for caveolin-1, caveolin-2, and rabbit polyclonal antibody specific for Ras. For comparison, lysates from cells transfected with plasmid pcDNA3.1 were analyzed (a,b). All antisera were used at a dilution of 1:1000.

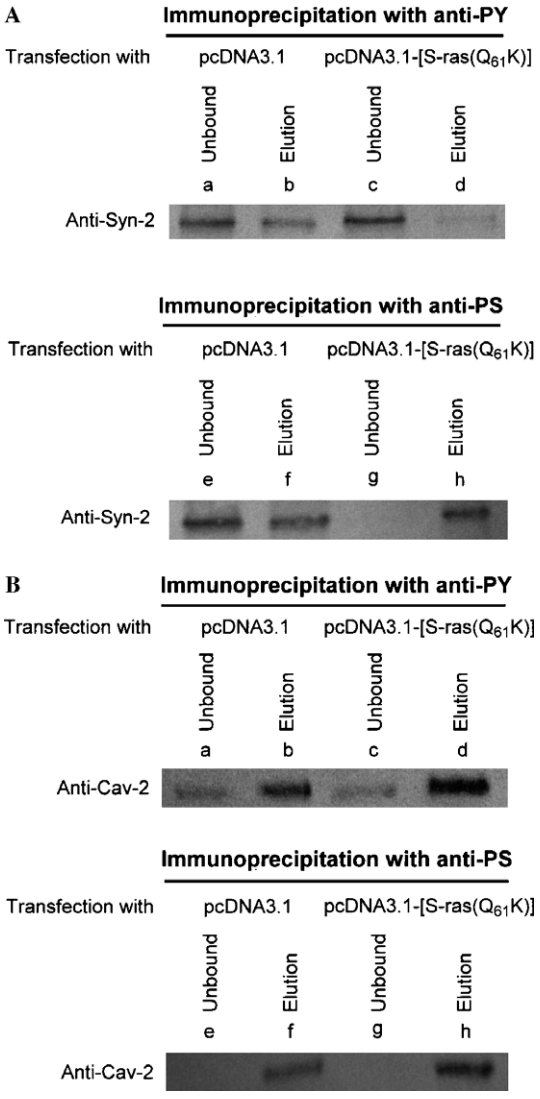


Fig. 2. Syndecan-2 is negligibly phosphorylated at tyrosine residues in cells transformed with oncogenic *ras*. (A) BALB/3T3 cells ($10^6$) transfected with plasmids of pcDNA3.1-[S-*ras*($Q_{61}$K)] were homogenized and membrane lysates were incubated with rabbit monoclonal antibody against phosphotyrosine (1 μg; a–d) or against phosphoserine (1 μg; e–h) at 37 °C for 30 min. The immunoprecipitates recovered in the elution buffer (100 μl) containing 25 mM Hepes, pH 7.5, and 1 M NaCl (d,h), and the previous unbound fractions (c,g) were subjected to electrophoresis on a Tricine–SDS–PAGE (12%), and Western blotting with rabbit polyclonal antibody specific for syndecan-2. For comparison, the lysates from cells transfected with plasmid pcDNA3.1 were analyzed (a, b, e, and f). (B) The lysates of BALB/3T3 cells ($10^6$) transfected with plasmids of pcDNA3.1-[S-*ras*($Q_{61}$K)] were incubated with rabbit monoclonal antibody against phosphotyrosine (1 μg; a–d) or against phosphoserine (1 μg; e–h) at 37 °C for 30 min. The immunoprecipitates recovered in the elution buffer (100 μl) containing 25 mM Hepes, pH 7.5, and 1 M NaCl (d,h) and the previous unbound fraction (c,g) were subjected to electrophoresis on a Tricine–SDS–PAGE (12%), and Western blotting with mouse monoclonal antibody specific for caveolin-2. For comparison, lysates from cells transfected with plasmid pcDNA3.1 were analyzed (a, b, e, and f). All antisera were used at a dilution of 1:1000.

anchor kinases and phosphatases for substrates. We thus investigated whether syndecan-2 in less tyrosine phosphorylated form as revealed in transformed cells interrupts the association with RACK1 and kinase.

### *$PY^{180}$ of syndecan-2 as a membrane anchor site for RACK1*

Expression of cDNA encoding human syndecan-2 *in vitro* yielded a core protein that migrated at 45 kDa (with a thioredoxin-tag of 150 amino acid residues) during Tricine–SDS–PAGE and that protein was phosphorylated by Src in the presence of [$\gamma$-$^{32}$P]ATP (Fig. 3B-a). We set out to mutate major tyrosine phosphorylation site $Y^{180}$ of syndecan-2 (syndecan-$2^{Y180F}$) and applied *in vitro* expressed syndecan-$2^{Y180F}$ to contrast the phosphorylation of $Y^{180}$ with Src. Autoradiography revealed that the radioactivity gain with syndecan-$2^{Y180F}$ was 80% less than with syndecan-2 (Fig. 3B-b). That is, $Y^{180}$ of syndecan-2 is a major phosphorylation site for Src.

We thus transfected cells with plasmids of syndecan-2 to confirm the tyrosine phosphorylation at $Y^{180}$ ($PY^{180}$) of syndecan-2 is important for binding with RACK1 *in vivo*. HeLa cells were applied in the study, because they were transfected successfully with plasmids containing syndecan-2 or syndecan-$2^{Y180F}$, syndecan-$2^{Y192F}$ or syndecan-$2^{Y180,192F}$ (with an efficiency of $>95\%$, respectively). The membrane lysates of transfected HeLa cells were immunoprecipitated with monoclonal antibodies against syndecan-2, challenged with non-specific phosphate competitor, sodium β-glycerophosphate, as well as with NaCl then analyzed the Western blot with anti-RACK1 (Fig. 3A). The treatment of sodium β-glycerophosphate to free phosphorylation-dependent reactants resulted in a significant detachment of RACK1 from the anti-syndecan-2 immune complex in cells transfected with syndecan-2 (Fig. 3A-a) or syndecan-$2^{Y192F}$ (Fig. 3A-c). In contrast, a neglected amount of RACK1 was eluted with sodium β-glycerophosphate from cells transfected with syndecan-$2^{Y180F}$ (Fig. 3A-b) and syndecan-$2^{Y180,192F}$(Fig. 3A-d).

Data indicate that selective phosphorylation at $Y^{180}$ (not $Y^{192}$) of syndecan-2 contributes a lot to the recruitment of RACK1, but not all. However, the reaction of syndecan-2 with RACK1 does not depend exclusively on the $PY^{180}$ of syndecan-2. Almost all of RACK1 from cells transfected with syndecan-$2^{Y180F}$ (Fig. 3A-f) and syndecan-$2^{Y180,192F}$ (Fig. 3A-h), as well as 50% of RACK1 from cells transfected with syndecan-2 (Fig. 3A-e) and syndecan-$2^{Y192F}$ (Fig. 3A-g), were eluted with NaCl. In other words, a mechanism other than tyrosine phosphorylation of syndecan-2 at $Y^{180}$ is required to shut off RACK1 completely from the attachment of syndecan-2 in response to oncogenic *ras*.

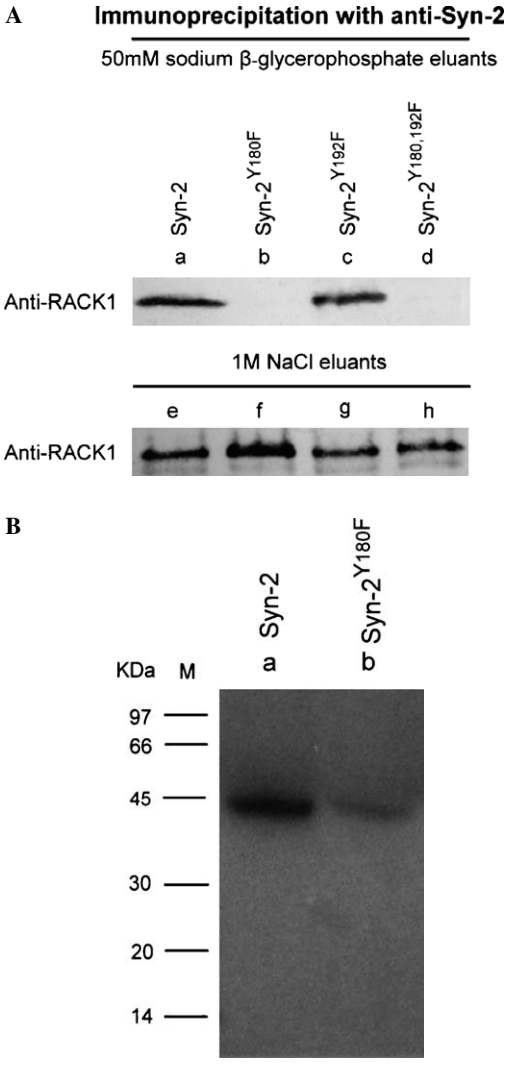


Fig. 3. Syndecan-2 protein reacts with RACK1. (A) HeLa cells ($10^6$) transfected with plasmids of syndecan-2 (a), syndecan-$2^{Y180F}$ (b), syndecan-$2^{Y192F}$ (c), and syndecan-$2^{Y180,192F}$ (d) were homogenized and membrane lysates were prepared as mentioned previously. The supernatants were incubated with rabbit polyclonal antibody against syndecan-2 (1 μg) at 37 °C for 30 min. The immunoprecipitate recovered in the eluant buffer (100 μl) containing 50 mM sodium β-glycerophosphate, pH 7.5 (a–d), and recovered consecutively in the buffer containing 25 mM Hepes, pH 7.5, and 1 M NaCl (e–h) was subjected to electrophoresis on a Tricine–SDS–PAGE (12%), and Western blotting with mouse monoclonal antibody specific for RACK1. The antiserum was used at a dilution of 1:1000. (B) The purified recombinant syndecan-2 protein (1 μg) and syndecan-$2^{Y180F}$ protein (1 μg) were incubated, respectively, with c-Src (Upstate; one unit) in the reaction buffer as mentioned in Materials and Methods, and the reaction was initiated by addition of 10 μCi of [$\gamma$-$^{32}$P]ATP. Phosphorylation was allowed to proceed for 10 min at 37 °C. Samples were subjected to electrophoresis on a Tricine–SDS–PAGE (12%) gel. The autoradiogram of the processed gel is shown.

### *Cytokine-responsive reactivity between syndecan-2 and RACK1*

Granylocyte-macrophage colony-stimulating factor (GM-CSF), a ligand for syndecan-2 [35] and a stimulator for tyrosine kinase JAK2 to phosphorylate syndecan-2 [36,37], was applied to find out whether the reaction between syndecan-2 and RACK1 is cytokine responsive. A time- and dose-dependent increase in the binding of RACK1 with syndecan-2 was found in the elution with sodium β-glycerophosphate (Fig. 4b–e) or NaCl

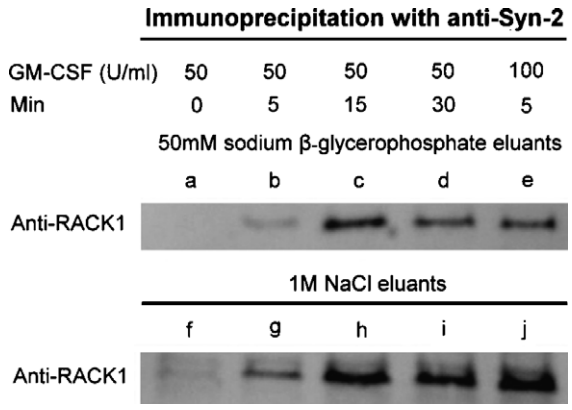


Fig. 4. Syndecan-2 reacts with RACK1 in GM-CSF dependency. HeLa cells ($10^6$) were treated with GM-CSF (50 or 100 U/ml) for different time intervals then homogenized and membrane lysates were incubated with rabbit polyclonal antibody against syndecan-2 (1 μg) at 37 °C for 30 min. The immunoprecipitate recovered in the eluant buffer (100 μl) containing 50 mM sodium β-glycerophosphate, pH 7.5 (a–e), and recovered consecutively in the buffer containing 25 mM Hepes, pH 7.5, and 1 M NaCl (f–j) were subjected to electrophoresis on a Tricine–SDS–PAGE (12%), and Western blotting with mouse monoclonal antibody specific for RACK1. The antiserum was used at a dilution of 1:1000.

(Fig. 4g–j). Huge amount of RACK1 to react with syndecan-2 was found with the treatment of GM-CSF at 50 U/ml for 15 min (Fig. 4c and h).

## Discussions

By transformation with oncogenic *ras* and analysis with immunoprecipitation, syndecan-2 was found to detach from RACK1 but associate with caveolin-2 and Ras in transformed cells. In parallel, syndecan-2 was identified in a tyrosine phosphorylated freed form in transformed cells, the tyrosine kinase is presumably inactive, in contrast with tyrosine phosphorylated form in cells without transformation. This seems unlikely on the basis of current knowledge of kinases in signal-transduction systems of mammalian cells, where kinase activity is believed to be transient. However, it cannot be ruled out that the accumulation of tyrosine phosphorylated syndecan-2 may reflect the accumulation of constitutively phosphorylated syndecan-2 in a silent molecular complex with RACK1 and kinases immobilized at caveolae as suggested recently [29,30]. The molecular complex of syndecan-2 is probably then committed to rapid dephosphorylation by an activated tyrosine phosphatase in cells transformed with oncogenic *ras*, to dislodge RACK1 and deprive PKC signals, to trap caveolin-2 at syndecan-2 and interrupt hetero-oligomerization with caveolin-1, thus to sweep out caveolae microdomains. The possibility of such a regulatory phosphotyrosyl protein phosphatase, as suggested recently to promote synapse growth [38], is currently under investigation in transformed cells. The data reinforce the view that post-translational modification of syndecan-2, such as tyrosine dephosphorylation, might result in a rearrangement of molecules in association with syndecan-2 and a shift of molecular associates from caveolae to non-raft, in contrast with tyrosine phosphorylation signals to shift syndecan-2 from non-raft to caveolae [16].

In the present study, the identity of the tyrosine kinase that phosphorylates syndecan-2 in BALB/3T3 cells is not known, but suggested to be JAK2 in the study with GM-CSF [35–37], or EphB2 receptor tyrosine kinase in neurospine formation [39]. By using cells transfected with plasmids of syndecan-2 mutants, $Y^{180}$ of syndecan-2 was demonstrated as a catalytic site for Src tyrosine kinase and $PY^{180}$ of syndecan-2 was revealed as an anchor site for RACK1. The $PY^{180}$ of syndecan-2 provides a binding site for the SH2 domains of scaffold protein RACK1, and thereby appears to upregulate PKC signaling [40] for GM-CSF signals or for left–right asymmetry pattern formation during embryogenesis [41]. However, in cells transfected with syndecan-$2^{Y180F}$, RACK1 maintains its reactions with syndecan-2 independent of phosphorylation. The possibility of complex mechanisms, such as molecular flow after syndecan-2 clustering [16] or caveolin polymerization, cannot be ignored. Further studies are directed towards uncovering the aberrant molecular reactions at caveolae during transformation.

## References

[1] J.L. Bos, *ras* oncogenes in human cancer, Cancer Res. 49 (1989) 4682–4689.
[2] D.R. Lowry, B.M. Willumsen, Function and regulation of *ras*, Annu. Rev. Biochem. 62 (1989) 851–891.
[3] M. Barbacid, *ras* genes, Annu. Rev. Biochem. 56 (1987) 779–827.
[4] G.L. James, J.L. Goldstein, M.S. Brown, Polylysine and CVIM sequences of K-RasB dictate specificity of prenylation and confer resistance to benzodiazepine peptidomimetic *in vitro*, J. Biol. Chem. 270 (1995) 6221–6226.
[5] S.A. Armstrong, M.C. Seabra, T.C. Sudhof, J.L. Goldstein, M.S. Brown, cDNA cloning and expression of the α and β subunits of rat Rab geranylgeranyltransferase, J. Biol. Chem. 268 (1993) 12221–12229.
[6] E.C. Lerner, Y. Qian, A.D. Hamilton, S.M. Sebti, Disruption of oncogenic K-Ras4B processing and signaling by a potent geranylgeranyltransferase I inhibitor, J. Biol. Chem. 270 (1995) 26770–26773.
[7] C.F. Huang, N.-N. Chuang, Facilitated geranylgeranylation of shrimp *ras*-encoded p25 fusion protein by the binding with guanosine diphosphate, J. Exp. Zool. 283 (1999) 510–521.
[8] K. Pienta, A.W. Partin, D.S. Coffey, Cancer as a disease of DNA organization and dynamic cell structure, Cancer Res. 49 (1989) 2525–2532.
[9] C.C. Lopes, L. Toma, M.A.S. Pinhal, M.A. Porcionatto, M.C. Sogayar, C.P. Dietrich, H.B. Nader, EJ-*ras*oncogene transfection of endothelial cells upregulates the expression of syndecan-4 and downregulates heparan sulfate sulfotransferases and epimerase, Biochimie (in press).
[10] A.C. Rapraeger, V.L. Ott, Molecular interactions of the syndecan core proteins, Curr. Opin. Cell Biol. 10 (1998) 620–628.
[11] M. Bernfield, R. Kokenyesi, M. Kato, M.T. Hinkes, J. Spring, R.L. Gallo, E.J. Lose, Biology of the syndecans: a family of transmembrane heparan sulfate proteoglycans, Annu. Rev. Cell. Biol. 8 (1992) 365–393.
[12] V.L. Ott, A.C. Rapraeger, Tyrosine phosphorylation of syndecan-1 and -4 cytoplasmic domains in adherent B82 fibroblasts, J. Biol. Chem. 273 (1988) 35291–35298.

[13] J.C. Adams, F.M. Watt, Regulation of development and differentiation by the extracellular matrix, Development 117 (1993) 1183–1198.
[14] B.M. Gumbiner, Cell adhesion: the molecular basis of tissue architecture and morphogenesis, Cell 84 (1996) 345–357.
[15] T. Kinnunen, M. Kaksonen, J. Saarinen, N. Kalkkinen, H.B. Peng, H. Rauvala, Cortactin-Src kinase signaling pathway is involved in N-syndecan-dependent neurite outgrowth, J. Biol. Chem. 273 (1998) 10702–10708.
[16] E. Tkacheno, M. Simons, Clustering induces redistribution of syndecan-4 core protein into raft membrane domains, J. Biol. Chem. 277 (2002) 19946–19951.
[17] K.G. Rothberg, J.E. Heuser, W.C. Donzell, Y.-S. Ying, J.R. Glenney, R.G.W. Anderson, Caveolin, a protein component of caveolae membrane coats, Cell 68 (1992) 673–682.
[18] P. Dupree, R.G. Parton, G. Raposo, T.V. Kurzchalia, K. Simons, Caveolae and sorting in the trans-Golgi network of epithelial cells, EMBO. J. 12 (1993) 1597–1605.
[19] S. Monier, R.G. Parton, F. Vogel, J. Behlke, A. Henske, T.V. Kurzchalia, VIP21-caveolin, a membrane protein constituent of the caveolar coat, oligomerizes in vivo and in vitro, Mol. Biol. Cell 6 (1995) 911–927.
[20] M. Way, R.G. Parton, M-caveolin, a muscle-specific caveolin-related protein, FEBS. Lett. 376 (1995) 108–112.
[21] P.E. Scherer, T. Okamoto, M. Chun, T. Nishimoto, H.F. Lodish, M.P. Lisanti, Identification, sequence, and expression of caveolin-2 defines a caveolin gene family, Proc. Natl. Acad. Sci. USA 93 (1996) 131–135.
[22] Z. Tang, P.E. Scherer, T. Okamoto, K. Song, C. Chu, D.S. Kohtz, I. Nishimoto, H.F. Lodish, M.P. Lisanti, Molecular cloning of caveolin-3, a novel member of the caveolin gene family expressed predominantly in muscle, J. Biol. Chem. 271 (1996) 2255–2261.
[23] D. Mochly-Rosen, B.L. Smith, C.H. Chen, M.H. Disatnik, D. Ron, Interaction of protein kinase C with RACK1, a receptor for activated C-kinase: a role in beta protein kinase C mediated signal transduction, Biochem. Soc. T. 23 (1995) 596–600.
[24] T. Pawson, J.D. Scott, Signaling through scaffold, anchoring, and adaptor proteins, Science 278 (1997) 2075–2080.
[25] J. Sondek, D.P. Siderovski, Gγ-like (GGL) domains: new frontiers in G-protein signaling and β-propeller scaffolding, Biochem. Pharmacol. 61 (2001) 1329–1337.
[26] Y.-H. Chen, H.-C. Wang, C.-Y. Lin, N.-N. Chuang, Effects of prenyl pyrophosphates on the binding of PKCγ with RACK1, J. Exp. Zool. 295A (2003) 71–82.
[27] M. Csukai, C.-H. Chen, M.A. DeMatteis, D. Mochly-Rosen, The coatomer protein β′-COP, a selective binding protein (RACK) for protein Kinase C εγρ, J. Biol. Chem. 272 (1997) 29200–29206.
[28] N.-N. Chuang, S.L. Shih, Purification and some properties of alkaline phosphatase from the hepatopancreas of the shrimp *Penaeus japonicus* (Crustacea: Decapoda), J. Exp. Zool. 256 (1990) 1–7.
[29] J.-W. Huang, C.-L. Chen, N.-N. Chuang, P120-GAP associated with syndecan-2 to function as an active switch signal for Src upon transformation with oncogenic *ras*, Biochem. Biophys. Res. Commun. 329 (2005) 855–862.
[30] J.-W. Huang, C.-L. Chen, N.-N. Chuang, Trap RACK1 with Ras to mobilize Src signaling at syndecan-2/p120-GAP upon transformation with oncogenic ras, Biochem. Bioph. Res. Co. 330 (2005) 1087–1094.
[31] C.-F. Huang, C.-H. Chen, N.-N. Chuang, Disrupting the transforming activity of shrimp *ras*($Q_{61}K$) by deleting the CAAX box at the C-terminus, J. Exp. Zool. 289 (2001) 441–448.
[32] H. Schagger, G. von Jagow, Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis, Anal. Biochem. 166 (1987) 368–379.
[33] L.C. Lane, A simple method for stabilizing protein-sulfhydrylgroups during SDS-gel electrophoresis, Anal. Biochem. 86 (1978) 655–664.
[34] C.R. Merril, D. Goldman, S.A. Sedman, M.H. Ebert, Ultrasensitive stain for proteins in polyacrylamide gel shows regional variation in cerebrospinal fluid proteins, Science 211 (1981) 1437–1438.
[35] D. Modrowski, M. Basle, A. Lomri, P.J. Marie, Syndecan-2 is involved in the mitogenic activity and signaling of granulocyte-macrophage colony-stimulating factor in osteoblasts, J. Biol. Chem. 275 (2000) 9178–9185.
[36] J. Gomez-Cambronero, C. Veatch, Emerging paradigms in granulocyte-macrophage colony-stimulating factor signaling, Life Sci. 59 (1996) 2099–2111.
[37] K. Okuda, J.S. Sanghera, S.L. Pelech, Y. Kanakura, M. Hallek, J.D. Griffin, B.J. Drucker, Granulocyte-macrophage colony-stimulating factor, interleukin-3, and steel factor induce rapid tyrosine phosphorylation of p42 and p44 MAP kinase, Blood 79 (1992) 2880–2887.
[38] K.G. Johnson, A.P. Tenney, A. Ghose, A.M. Duckworth, M.E. Higashi, K. Parfitt, O. Marcu, T.R. Heslip, J.L. Marsh, T.L. Schwarz, J.G. Flanagan, D. Van Vactor, The HSPGs syndecan and Dallylike bind the receptor phosphatase LAR and exert distinct effects on synaptic development, Neuron 49 (2006) 517–531.
[39] I.M. Ethell, F. Irie, M.S. Kalo, J.R. Couchman, E.B. Pasquale, Y. Yu, EphB/syndecan-2 signaling in dendritic spine morphogenesis, Neuron 31 (2001) 1001–1013.
[40] E.-S. Oh, A. Woods, J.R. Couchman, Syndecan-4 proteoglycan regulates the distribution and activity of protein kinase C, J. Biol. Chem. 272 (1997) 8133–8136.
[41] K.L. Kramer, J.E. Barnette, H.J. Yost, PKCγ regulates syndecan-2 inside-out signaling during*Xenopus*left-right development, Cell 111 (2002) 981–990.