Galectin-1(L11A) Predicted from a Computed Galectin-1 Farnesyl-Binding Pocket Selectively Inhibits Ras-GTP

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ABSTRACT

Ras biological activity necessitates membrane anchorage that depends on the Ras farnesyl moiety and is strengthened by Ras/galectin-1 interactions. We identified a hydrophobic pocket in galectin-1, analogous to the Cdc42 geranylgeranyl-binding cavity in RhoGDI, possessing homologous isoprenoid-binding residues, including the critical L11, whose RhoGDI L77 homologue changes dramatically on Cdc42 binding. By substituting L11A, we obtained a dominant interfering galectin-1 that possessed normal carbohydrate-binding capacity but inhibited H-Ras GTP-loading and extracellular signal-regulated kinase activation, dislodged H-Ras(G12V) from the cell membrane, and attenuated H-Ras(G12V) fibroblast transformation and PC12-cell neurite outgrowth. Thus, independently of carbohydrate binding, galectin-1 cooperates with Ras, whereas galectin-1(L11A) inhibits it.

INTRODUCTION

Ras proteins regulate diverse effectors that control cell proliferation, differentiation, survival, and death (1–5). Anchorage of Ras to cell membranes is vital for its biological activities (6–8). Exchange of GDP for GTP promotes activation by Ras of its effectors, and its activity is terminated by GTPase-activating proteins that facilitate GTP hydrolysis (9–11). The strength, duration, and selectivity of the Ras signal is also determined by the spatio-temporal organization of Ras in the plasma membrane and other internal membranes (12–18).

Recent studies showed that galectin-1, a β-galactoside-binding protein (19–22), interacts with H-Ras-GTP, strengthening its membrane association (23). H-Ras-GTP recruits galectin-1 from the cytosol to the cell membrane, with resulting stabilization of H-Ras-GTP (23), clustering of H-Ras-GTP and galectin-1 in nonraft microdomains (24), enhancement of the Ras signal to extracellular signal-regulated kinase (ERK), and increased cell transformation (23, 25). These observations indicate the significance of Ras/galectin-1 interactions in Ras biology.

The mode of H-Ras-galectin-1 interaction at the molecular level is still unresolved. A clue to a possible mechanism of interactions that involve the farneslycysteine of Ras comes from the observation that N-myristoylated unfarnesylated H-Ras(12V) does not interact with galectin-1 (23). We thus assumed that certain structural traits, especially spatial clustering of hydrophobic amino acid side chains in galectin-1, might accommodate the farnesyl group of Ras. Here, we identified a hydrophobic pocket in galectin-1 and showed that substitution of a single amino acid residue within this pocket (“L11A substitution”) generated a dominant interfering galectin-1 that inhibits active H-Ras.

MATERIALS AND METHODS

Modeling. We used Insight II software (Accelrys, San Diego, CA) for structural comparisons, superimposition, and Connolly surface analysis of galectin-1, RhoGDI, and the RhoGDI/Cdc42 complex (Protein Data Bank codes 1SLA, 1RHO, and 1DOA, respectively) and for graphical presentations. Sequence alignments were performed using the Pileup software (Accelrys). To estimate the surface contact surface areas of amino acid residues in RhoGDI interacting with the geranylgeranyl moiety, the IDOA data were analyzed by the Ligand-Protein Contacts software (26).

Expression Vectors. pcDNA1, pcDNA-galectin-1, antisense, Ras, and green fluorescent protein (GFP)-Ras vector constructs have been described previously (15, 23, 27). The galectin-1 mutant (L11A) was generated by PCR using the primers 5’-gggagctcatggctgtgtgtgctgagccagactc-3’ and 5’-tactacaaaggtcacacagtgcggg-3’. The PCR product was subcloned into the pcEGFT-EAZY vector (Promega) and then cloned into the NorI site of pcDNA3, and the sequence was verified. Recombinant protein expression vectors for rat galectin-1 and galectin-1(L11A) were prepared by PCR amplification. The amplicon was ligated into the EcoRV-linearized pET-Blue-1 AccpTor Vector (Novagen, Darmstadt, Germany) and propagated for recombinant protein expression in E. coli strain Tuner(DE3)pLacI.

Protein Purification and Characterization. Wild-type (wt) and mutant proteins were purified from extracts by affinity chromatography on lactosyl-Sepharose 4B as described (28). We used one- and two-dimensional gel electrophoresis to assess purity of the proteins, which were then characterized by gel filtration using a Superose 12 HR 10/30 column calibrated with standard proteins (Bio-Rad, Munich, Germany) and human galectin-1 as described (28). Hemagglutination assays with trypsin-treated and glutardialdehyde-fixed rabbit erythrocytes were performed as described (29). The carbohydrate-dependent binding of biotinylated galectin-1 to surface-immobilized asialofetuin was assessed in solid-phase assays, as described (30).

Coimmunoprecipitation and Western Immunoblotting. We cotransfected 1.5 × 10⁵ COS-7 cells using dextran (Pharmacia, Stockholm, Sweden) with 0.75 μg of plasmid DNA coding for galectin-1 or 3 μg of plasmid DNA coding for galectin-1(L11A), together with 0.75 μg of plasmid DNA coding for H-Ras(G12V) or H-Ras(wt). The amount of DNA was complemented to a total of 4.5 μg by pcDNA3. The transfected cells were lysed 48 h later. Lysates were subjected to immunoprecipitation with mouse pan-Ras antibody (Ab-3; Calbiochem) followed by SDS-PAGE and Western blotting as described (25) with either pan anti-Ras Ab and peroxidase-goat antimouse IgG (The Jackson Laboratory, Bar Harbor, ME) or anti-galectin-1 Ab and peroxidase-goat anti-rabbit IgG (The Jackson Laboratory) as described previously (23, 25). We visualized Ras proteins and the M₇, 14,000 galectin-1 protein by enhanced chemiluminescence and quantified the bands by densitometry (normalized arbitrary units; Ref. 25) with Image Master VDS-CL (Amer sham Pharmacia Biotech, Piscataway, NJ) using TINA 2.0 software (Ray Tests).

Subcellular Fractionation and Ras-GTP and Phospho-ERK Assays. We cotransfected 8 × 10⁵ HEK-293 cells using calcium phosphate (Promega, Madison, Wisconsin, Germany), a combination of 0.15 μg of plasmid DNA coding for GFP-H-Ras [or H-Ras or H-Ras(G12V)] and 5.85 μg of empty pcDNA3, 2.85 μg of plasmid DNA coding for galectin-1 and 3 μg of pcDNA3, or 5.85 μg of plasmid DNA coding for galectin-1(L11A). Untagged Ras-cotransfectants were used 48 h after transfection for subcellular fractionation (100,000 × g pellet, P100, and supernatant, S100), followed by SDS-PAGE and immunoblotting, as described (25). GFP-H-Ras cotransfectants were serum starved 24 h after transfection for 24 h. The cells were then stimulated with 100 ng/ml epidermal growth factor (EGF) for the indicated times and then lysed and assayed for Ras-GTP and phospho-ERK by immunoblotting, as described (23, 25). Anti-ERK1/2 Ab (Santa-Cruz Biotechnology, Santa Cruz, CA) and antiphospho-ERK Ab (Sigma-Aldrich, St. Louis, MO) were used.

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Confocal Microscopy and Colocalization. We plated 2.5 × 10^5 COS-7 cells on glass coverslips and then cotransfected the cells with 0.3 μg of plasmid DNA coding for galectin-1, galectin-1(L11A), or antisense galectin-1 RNA, together with 0.2 μg of plasmid DNA coding for GFP-H-Ras(G12V) or GFP-H-Ras, as described (15, 23, 27). The transfected cells were fixed or permeabilized and both labeled with rabbit galectin-1 Ab (23), followed by incubation with biotin-goat antirabbit IgG and Cy3-streptavidin (Jackson ImmunoResearch Laboratories, West Grove, PA), as described (23). The cells were then analyzed with a Zeiss LSM 510 confocal microscope fitted with nonleaking green and red fluorescence filters, and colocalization was assessed by the colocalization function of the LSM 510 software, as described (27, 31).

Transformation and Neurite Outgrowth Assays. Transformation was assayed as described (23, 32). Briefly, 8 × 10^5 EJ cells were transfected (calcium phosphate method) with 12 μg of pcDNA3 or 12 μg of pcDNA3-galectin-1(L11A). The medium was replaced, 72 h after transfection, by a selection medium containing 1.2 mg/ml G418, and the cells were kept in this medium for 10 days. They were then fixed, stained with 0.1% crystal violet, and analyzed microscopically. The numbers and sizes of foci were assessed using Image-Pro Plus software (Media Cybernetics). PC12 cells (5 × 10^5) plated on collagen-coated coverslips were transfected by Lipofectamine (Life Technologies, Inc., Rockville, MD) with 1 μg of pEGFP or cotransfected with 1 μg of pEGFP-H-Ras(G12V), together with 2 μg of pcDNA3, 1 μg of pcDNA3-galectin-1 and 1 μg of pcDNA3, or 2 μg of pcDNA3-galectin-1(L11A). The cells were fixed, 48 h after transfection, with 4% paraformaldehyde and subjected to analysis by fluorescence microscopy. Numbers and lengths of neurites were assessed using Image-Pro Plus software.

RESULTS

Structural Convergence among the Geranylgeranyl-Binding Domain of RhoGDI and Galectin-1. To identify a putative farnesyl-binding site in galectin-1, we used the Cdc42-RhoGDI complex as a template (33). The RhoGDI immunoglobulin-like domain-folding pattern and galectin-1 jelly roll-like fold (34) show similarities in their global secondary structures (Fig. 1A). Both constitute a β-sandwich, which in RhoGDI, forms a hydrophobic cavity hosting the geranylgeranyl moiety of Cdc42 (Ref. 33; Fig. 1A). We assumed that, as in RhoGDI, a set of hydrophobic amino acids in the galectin-1 sequence must be suitably positioned to confer isoprenoid-binding capacity. We ran multiple sequence alignments with RhoGDI and mammalian galectins and found that 10 of the 13 amino acid residues of Rho-GDI that interact with the geranylgeranyl group (33) either align perfectly with identical amino acids of rat and human galectin-1 (six residues) or are subject to conservative changes (four residues; Fig. 1B). The aligned amino acids face an interior region of galectin-1 residing between the two β-sheet layers of the protein, similarly to the way in which they face the inner surface of the immunoglobulin domain in Rho-GDI (Fig. 1C). Hence, the aligned residues may have a common isoprenoid-binding function.

We therefore carried out a computational structural analysis, in which the relevant parts of galectin-1 (34) were superimposed on RhoGDI in its Cdc42-bound form (Ref. 33; see “Materials and Methods”). The analogous amino acids, shown by the sequence alignment analysis to be strictly identical, were used as anchors. By this method, the positions of L9, L11, L17, F30, F32, and I128 of galectin-1 were compared with the corresponding positions in RhoGDI (35) and RhoGDI/Cdc42 complex (33) and galectin-1 (34) in ribbon and β-strand presentations. B, sequence alignment of rat and human galectin-1 and bovine RhoGDI. Red, identical amino acid residues are shown; blue, homologous residues. C, the aligned homologous amino acid residues of RhoGDI and galectin-1 face the interior of the β-sandwich of the two proteins. The structures of the RhoGDI immunoglobulin-like domain in its Cdc42-bound form and galectin-1 are shown in a β-strand representation and the important amino acids, as well as the geranylgeranyl group in a stick presentation. The illustration of the RhoGDI immunoglobulin-like domain with the inserted geranylgeranyl moiety of Cdc42 (its protein part is not shown) is based on the X-ray structure of the complex (33). The structure of the geranylgeranyl-bound, RhoGDI immunoglobulin-like domain was superimposed on the structure of one subunit of homodimeric galectin-1 as described in the text, resulting in the observed position of the isoprenoid group within the β-sandwich (the structures of only galectin-1 and the geranylgeranyl moiety are shown).
interaction. To address this issue, we scrutinized the data on the galectin-1 atomic temperature factor (B-factor), a characteristic quantified by X-ray analysis of the protein's structure (34). The B-factor is high for flexible atoms and low for rigid sections of the crystallized protein (36). We found that the loops in the putative area of insertion of the isoprenoid moiety into galectin-1 had a relatively high temperature factor compared with other loops (Fig. 2A), suggesting that the hydrophobic cavity can increase in size on binding of the farnesyl group of Ras.

**Design of Galectin-1(L11A) Mutant.** Next, we challenged our model by site-directed mutagenesis aimed at impairing the galectin-1/H-Ras interactions. We judged that it should be feasible to impair the capacity of isoprenoid binding by targeting the amino acid whose side chain ranks first in contact surface area. Assessment of the contact areas (see “Materials and Methods”) of the six geranylglyceranyl-binding residues of RhoGDI with identical counterparts in galectin-1 indicated that L77 was the first choice, because it exhibited the largest contact surface area with the geranylglyceranyl group; the respective contact surface areas of L75, L77, L88, L102, L104, and L177 were (Å²) 32.3, 41.1, 7.9, 23.1, 8.1, and 5.2. Moreover, L77 is a key player in the dramatic structural change of the geranylglyceranyl-binding cavity of RhoGDI on the formation of the Cdc42/RhoGDI complex (33). The sequence alignment and computational superimpositions suggested that L11 in galectin-1 would have a positional role equivalent to that of L77 in RhoGDI (Fig. 1). We therefore performed L11A substitution in galectin-1 and used the mutant galectin-1 to test the validity of our concept.

**Mutant Galectin-1(L11A) Retains Major wt Characteristics.** Gel filtration analysis (28) of galectin-1 and galectin-1(L11A) showed no difference (17.64 and 17.59 min, respectively) between the retention times of the two proteins, indicating that the mutation had no detectable impact on dimerization or shape. This was confirmed by hemagglutination assays (37); the minimal lectin concentration required to yield hemagglutination was 0.15 μg/ml for both galectin-1 and galectin-1(L11A). The hemagglutination assay also permits determination of carbohydrate-binding capacity in a semiquantitative manner, as assessed by the inhibition of hemagglutination with serial dilutions of the haptenic sugar lactose (38). We found only a small difference between the lactose concentrations, resulting in 50% inhibition of hemagglutination by galectin-1 and galectin-1(L11A)(1.25 and 2.5 mM, respectively). We then used an accurate solid-phase, carbohydrate-binding assay with asialofetuin as the glycoprotein ligand (38) and found that galectin-1 and galectin-1(L11A) exhibited saturable and similar binding activities (Fig. 3). Taken together, these experiments showed that the L11A substitution in galectin-1 did not cause distortion of the dimer status/shape or the carbohydrate-binding activity.

![Fig. 2. Identification of a putative isoprenoid insertion site in galectin-1 as a flexible hydrophobic cavity. The conserved elements of the structure of the geranylglyceranyl-bound RhoGDI immunoglobulin fold were superimposed separately on the structure of the ligand-free RhoGDI immunoglobulin-like domain (33) or jelly roll-like fold of one protomer of galectin-1 (34), and the position of the isoprenoid group observed within the depicted structures is illustrated by superimpositions to highlight the emerging similar theme (see “Materials and Methods”). A, Connolly surface analysis of the superimposed structures, with emphasis on the position of the geranylglyceranyl group. Structural details of the RhoGDI/Cdc42 complex are deliberately omitted. B, ribbon representation of galectin-1, colored on the basis of the numerical value of the B factor (details in the text). Color scale: red, high values; blue, low values.

![Fig. 3. Carbohydrate-binding activities of galectin-1 and galectin-1(L11A). Representative curves of the specific binding of galectin-1(wild type) (A) and galectin-1(L11A) (B) to surface-exposed asialofetuin are shown (insets). Main frames depict Scatchard plots of the binding. The dissociation constants (Kd) and extent of bound probe molecules at saturation (Bmax) thus calculated (means ± SD, n = 4) were: Kd = 30.3 ± 10.8 and 49.9 ± 27 nm and Bmax = 4.2 ± 1.3 and 5.9 ± 2.3 × 10¹¹ bound probe molecules for galectin-1 and galectin-1(L11A), respectively.](image)
Galectin-1(L11A) Coimmunoprecipitates with H-Ras(G12V) and Is Exclusively Localized to the Cytosol. Using coimmunoprecipitation assays, we next examined the capacity of galectin-1(L11A) to interact with H-Ras. Ras proteins were immunoprecipitated with anti-Ras Ab from extracts of HEK-293 cells cotransfected with H-Ras(G12V) and galectin-1 or H-Ras(G12V) and galectin-1(L11A). The immunoprecipitated proteins were then immunoblotted with an antibody to galectin-1. C, subcellular distribution of galectin-1(L11A) and galectin-1; D, subcellular distribution of H-Ras(wild type).

Galectin-1(L11A) Inhibits the EGF-Stimulated GFP-H-Ras(G12V) Colocalization in the Cell Membrane. Next, we examined the extent of colocalization of GFP-H-Ras(G12V) and galectin-1(L11A) in the cell membrane. COS-7 cells were cotransfected with galectin-1(L11A) and H-Ras(G12V) or H-Ras(G12V)/galectin-1(L11A). The cells were fixed, and confocal fluorescence images were collected. Typical images (depicted in Fig. 5, A–C) show that unlike in the GFP-H-Ras(G12V) transfectants, in which GFP-H-Ras(G12V) was strongly localized to the cell membrane, in the GFP-H-Ras(G12V)/galectin-1(L11A) cotransfectants, GFP-H-Ras(G12V) was localized mainly in submembrane cytosolic pools. Thus, galectin-1(L11A) induced mislocalization of GFP-H-Ras(G12V) in a manner reminiscent of GFP-H-Ras(G12V) mislocalization induced by galectin-1 antisense RNA (Ref. 23; Fig. 5).

GFP-H-Ras(G12V) Colocalizes with Galectin-1 but not Galectin-1(L11A) in the Cell Membrane. Next, we examined the extent of colocalization of GFP-H-Ras(G12V) and galectin-1(L11A) in the cell membrane. COS-7 cells were cotransfected with galectin-1 or galectin-1(L11A) and GFP-H-Ras(G12V) or GFP-H-Ras(wt). Galectin-1 or galectin-1(L11A) was labeled with anti-galectin-1 Ab and then with biotinylated goat antirat IgG and streptavidin-Cy3. Dual fluorescence images (GFP, green; Cy3, red) were recorded and stored, overlaid, and examined for colocalization using the colocalization function of the confocal imaging program. Fluorescence profiles of typical cells in these experiments are shown in Fig. 5, D–R. In galectin-1/GFP-H-Ras(G12V); Fig. 5, D–F) but not galectin-1/L11A-GFP-H-Ras(wt) (Fig. 5, J–L) cotransfectants, a significant fraction of the galectin-1 population was colocalized with H-Ras(G12V) at the rim of the cells, resulting in the typical plasma membrane labeling. This is consistent with the observation that H-Ras(G12V) expression increased the amount of galectin-1 in the particulate fraction (Fig. 4B). By contrast, in GFP-H-Ras(G12V)/galectin-1(L11A); Fig. 5, G–I) but not GFP-H-Ras(wt)/galectin-1(L11A); Fig. 5, M–O) cotransfectants, both GFP-H-Ras(G12V) and galectin-1(L11A) were localized to the cytosol without significant colocalization at the cell membrane. These findings showed that galectin-1 is attracted to the cell membrane by H-Ras(G12V) but not H-Ras(wt), whereas galectin-1(L11A) appears to attract H-Ras(G12V), but not H-Ras(wt), to the cytosol.

Galectin-1(L11A) Inhibits the EGF-Stimulated GFP-H-Ras GTP Loading and Increase in Phospho-ERK and Attenuates the Biological Activity of H-Ras(G12V). We then examined the effect of galectin-1(L11A) on EGF-stimulated GTP loading of GFP-H-Ras(wt) in GFP-H-Ras/galectin-1 or GFP-H-Ras/galectin-1(L11A) cotransfectants, using a Ras-GTP pull-down assay (25). Compared with control GFP-H-Ras transfectants, the EGF-stimulated increase in Ras-GTP in GFP-H-Ras/galectin-1 cotransfectants was 2-fold higher and lasted longer (Ref. 25; Fig. 6A). By contrast, in GFP-H-Ras/galectin-1(L11A) cotransfectants, the EGF-stimulated increase in GFP-H-Ras-GTP was far lower than that observed in GFP-H-Ras(wt) transfectants (Fig. 6A). These findings indicate that galectin-1(L11A) acts as an inhibitor of the active H-Ras protein.
We then examined the effect of galectin-1(L11A) on the EGF-stimulated H-Ras activation of ERK. The EGF-stimulated increase in active phospho-ERK in GFP-H-Ras/galectin-1 cotransfectants was higher and lasted longer than that in GFP-H-Ras transfectants (Fig. 6B). By contrast, in GFP-H-Ras/galectin-1(L11A) cotransfectants, the EGF-stimulated increase in phospho-ERK was significantly lower than that observed in GFP-H-Ras transfectants (Fig. 6B). Thus, the observed inhibitory effect of galectin-1(L11A) on EGF-stimulated GFP-H-Ras-GTP loading was also manifested in the Ras signal to the Raf/MEK/ERK pathway.

These results suggested that galectin-1(L11A) might inhibit the biological activity of H-Ras(G12V). To examine this possibility, we analyzed the effects of galectin-1(L11A) on two well-known activities of H-Ras(G12V), namely transformation in immortal rodent fibroblasts (32) and induction of neurite outgrowth in PC12 cells (39). In the first assay, we examined foci formation in galectin-1(L11A)- and vector-transfected H-Ras-transformed Rat-1 (EJ) cells in which galectin-1 is up-regulated because of overexpression of H-Ras(G12V) (23). Galectin-1(L11A) inhibited foci formation in EJ cells (Fig. 7A), as shown by the reduction in both number and size of the foci (Fig. 7A).

In the second assay, we used PC12 cells, which express relatively low levels of galectin-1.3 The cells were transfected with GFP (vector

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3 Unpublished observations.
DISCUSSION

In this study, we generated the dominant negative galectin-1(L11A), which acts as an inhibitor of Ras. Unlike galectin-1(wt), which cooperates with active Ras in strengthening H-Ras-GTP loading, membrane association, and cell transformation (23, 25), galectin-1(L11A) destabilizes Ras-GTP and its membrane association (Fig. 5) and inhibits Ras-dependent cell transformation or neurite outgrowth (Fig. 7). Although galectin-1(L11A) and galectin-1(wt) have markedly different effects on active Ras biology, they exhibit similar carbohydrate-binding activities and homodimerization properties (Fig. 3). These findings provide additional compelling evidence that intracellular galectin-1/Ras interactions are independent of galectin-1 carbohydrate-binding activities (23). The hydrophobic pocket in galectin-1 identified here, which is analogous to the Cdc42 geranylgeranyl-binding cavity in RhoGDI, harbors typical isoprenoid-binding residues, including the critical L11, whose L77 analogue in RhoGDI undergoes dramatic positional changes on Cdc42 binding (33). Our proposed model of insertion of the farnesyl group of Ras into this pocket in galectin-1 is supported by the observations that the single L11A mutation resulted in conversion of galectin-1 from a positive regulator of H-Ras (23, 25) to a strongly interfering protein of H-Ras-GTP. Whether H-Ras/galectin-1 interactions indeed involve insertion of the farnesyl moiety of Ras into the described hydrophobic pocket in galectin-1 will need to be validated by X-ray crystallography. Crystallographic descriptions of the COOH-terminal domain of Ras proteins or fully processed Ras in complex with its interacting proteins have not yet been achieved.

Galectin-1 is not the only Ras-binding protein in which a RhoGDI-like hydrophobic pocket has been identified. Recent structural studies of the cGMP phosphodiesterase 5-subunit (PDEδ) revealed a close structural similarity between PDEδ and RhoGDI (40, 41). Scrutiny of the superimposed structures of PDEδ and RhoGDI led to the identification of a hydrophobic pocket in PDEδ, proposed to be the putative binding site of the farnesyl isoprenoid moiety of H-Ras (40). PDEδ binds in vitro H-Ras(wt) but not H-Ras(G12V; Ref. 40). This and other data suggested that PDEδ might participate in transport of H-Ras to the cell membrane (40, 41). Unlike galectin-1, which belongs to the family of proteins harboring the jelly roll-like fold, PDEδ shares notable structural similarity with the immunoglobulin-like fold in RhoGDI (40, 41). These differences might be related to the preferential interactions of both RhoGDI and PDEδ with the GDP-bound forms of their partners, whereas galectin-1 and galectin-1(L11A) preferentially interact with H-Ras-GTP. In keeping with our earlier observations that galectin-1 can bind both active H-Ras and K-Ras and confers on both similar properties with respect to GTP stabilization and signal selectivity (25), preliminary results indicate that galectin-1(L11A) also inhibits the active form of K-Ras. Together, these data are consistent with our model that galectin-1 or galectin-1(L11A) bind to a site common to H-Ras and K-Ras, namely, the farnesyl cysteine carboxymethyl ester.

The way in which galectin-1(L11A) exerts its dominant inhibitory action on activated H-Ras is not yet known. The mechanism might involve a galectin-1(L11A)-induced conformational change in H-Ras-GTP protein, analogous to but distinct from that induced by galectin-1 in H-Ras-GTP. Binding of H-Ras-GTP to galectin-1 induces a Ras conformer that mimics H-Ras(G12V/T35S), which is locked in a conformation that promotes activation of Raf-1 but not phosphatidylinositol-4,5-bisphosphate (PIP2) activation. The detailed molecular mechanisms underlying these differences are being investigated. However, our studies indicate that galectin-1(L11A) binds with PDEδ to a site common to H-Ras and K-Ras, namely, the farnesyl cysteine carboxymethyl ester.
linositol 3’-kinase (25). Whether or not this is the case, the high selectivity of galectin-1(L11A) toward the activated Ras protein with no apparent effect on GDP-bound wt Ras suggests that galectin-1(L11A) might be a new type of Ras inhibitor that can be used for the design of novel anticancer drugs.

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