Cyclin-dependent Kinase Inhibitors and JNK Act as Molecular Switches, Regulating the Choice between Growth Arrest and Apoptosis Induced by Galectin-8*

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Galactin-8, a mammalian β-galactoside binding lectin, functions as an extracellular matrix protein that forms high affinity interactions with integrins. Here we demonstrated that soluble galectin-8 inhibits cell cycle progression and induces growth arrest. These effects cannot be attributed to interference with cell adhesion but can be attributed to a 4–5-fold increase in the cellular content of the cyclin-dependent kinase inhibitor p21, which was already evident following a 4-h incubation of H1299 cells with galectin-8. The increase in p21 levels was preceded by a 3–5-fold increase in JNK and protein kinase B (PKB) activities. Accordingly, SP600125, the inhibitor of JNK, and wortmannin, the inhibitor of phosphatidylinositol 3-kinase, which is the upstream activator of PKB, inhibited the increase in the cellular content of p21. Furthermore, overexpression of a dominant inhibitory form of SEK1, the upstream kinase regulator of JNK, inhibited both JNK activation and p21 accumulation. When p21 expression was inhibited by cycloheximide, galectin-8 directed the cells toward apoptosis, which involves induction of poly(ADP-ribose) polymerase cleavage. Indeed, galectin-8-induced apoptosis was 2-fold higher in HTIC (p21-null) cells when compared with parental HTC cells. Because overexpression of galectin-8 attenuates the rate of DNA synthesis, stable colonies that overexpress and secrete galectin-8 can be generated only in cells overexpressing a growth factor receptor, such as the insulin receptor. These results implicate galectin-8 as a modulator of cellular growth through up-regulation of p21. This process involves activation of JNK, which enhances the synthesis of p21, combined with the activation of PKB, which inhibits p21 degradation. These effects of the lectin depended upon protein-sugar interactions and were induced when galectin-8 was present as a soluble ligand or when it was overexpressed in cells.

The proliferation of animal cells is a highly conserved process tightly controlled by the interplay between growth-promoting and growth-limiting signals, the operation of which results in a timed progression through the cell cycle (1, 2). Signals that limit cell cycle advance are critically important for the control of cell number and the maintenance of tissue homeostasis both through restraints on cell proliferation and through the induction of programmed cell death (3, 4). The molecular machinery that controls cell cycle progression is based on the sequential activity of a family of protein kinases known as cyclin-dependent kinases (4). Considering the importance of cyclin-dependent kinases in cellular proliferation, it is not surprising that their activity is exquisitely regulated. One of the key players in this process is the cyclin-dependent kinase inhibitor p21 (also known as Waf1 or Cip1) (5). This protein and its related counterparts, p27 and p57, block progression through the cell cycle at the G1/S and G2/M checkpoints by forming ternary complexes with cyclin-dependent kinases, thus inhibiting their enzymatic activity (6). Cyclin-dependent kinase inhibitors are induced in response to DNA damage through the action of the tumor suppressor protein p53 (7). Growth factors up-regulate p21 in some cells, but this occurs in a p53-independent manner (8). Other receptor-ligands can also act as negative regulators of cell cycle progression. These include several cytokines such as Fas ligands (9), interferons (10), transforming growth factor β (11), and mammalian lectins termed galectins (12–14).

Galectins are defined by two properties, namely shared amino acid motifs and an affinity for β-galactoside-containing glycoconjugates (15–17). Although lacking a signal peptide and found mainly in the cytosol, galectins are externalized by an atypical secretory mechanism to mediate cell growth, cell transformation, embryogenesis, and apoptosis (reviewed in Refs. 18–21). In the present study we set out to determine the molecular elements involved in the growth regulation modulated by galectin-8, a family member of the galectins. Galectin-8 is a widely expressed and secreted protein made up of two homologous carbohydrate recognition domains joined by a short linking peptide (22, 23). It functions as an extracellular matrix protein, whose binding to cell surface integrins modulates cell adhesion and spreading upon the formation of high affinity interactions with integrins (24, 25). These interactions trigger a unique repertoire of signaling cascades that include sustained activation of downstream effectors of the MAPK and PI3K pathways. These effectors mediate the distinctive cytoskeletal organization that is induced when cells adhere, spread, and migrate on immobilized galectin-8 (26, 27). In contrast, transfection of galectin-8 cDNA into human lung carcinoma cells markedly inhibits colony formation, suggesting that the overexpressed galectin-8 may act as an inhibitor of cell...
growth (24). Support for this idea is provided by the fact that other galectins were implicated as negative regulators of cell cycle progression (13, 14, 28). However, the molecular basis for the growth inhibitory effects of galectins in general and galectin-8 in particular remains largely obscure.

The results presented here provide evidence that soluble galectin-8 inhibits cellular growth by activating both Jun kinase (JNK) and protein kinase B (PKB, also known as Akt), which promote the accumulation of the cyclin-dependent kinase inhibitor p21. Furthermore, when the expression of p21 is inhibited, galectin-8 drives the cells into an apoptotic process. These results implicate soluble galectin-8 as a potential modulator of cell growth through the up-regulation of genes encoding for inhibitors of cell cycle progression.

**EXPERIMENTAL PROCEDURES**

**Materials**—Bacterially expressed recombinant galectin-8 and glutathione S-transferase-galectin-8 were generated as described previously (22). Galectin-8 (2–4 mg/ml) was maintained in 30% glycerol in PBS and diluted in PBS to the final concentration as indicated. Glycerol-buffered diluted accordingly, served as the control. Restriction enzymes were purchased from New England Biolabs. Protease inhibitor mixture, insulin, lactosyl-agarose beads, glutathione-agarose beads,wortmannin, purnycomycin, cycloheximide, thiodigalactoside (TDG), and paraformaldehyde were from Sigma. Bromodeoxyuridine (BrdUrd), G418 sulfate, and SB203580, inhibitors of cell cycle progression (13, 14, 28). However, the molecular basis for growth arrest and apoptosis induced by galectin-8

The cDNA encoding full-length rat galectin-8 (the pGex-2T plasmid (22) was digested with BamHI and EcoRI, which promote the accumulation of the cyclin-dependent kinase inhibitor p21. Furthermore, when the expression of p21 is inhibited, Galectin-8 drives the cells into an apoptotic process. These results implicate soluble galectin-8 as a potential modulator of cell growth through the up-regulation of genes encoding for inhibitors of cell cycle progression.

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cleavage. Cell extracts were prepared in buffer A, and the proteins were resolved by means of SDS-PAGE (10% gels), transferred to a nitrocellulose membrane, and subjected to Western immunoblotting with anti-PARP antibodies.

**Real Time PCR—Adherent H1299 cells were incubated in serum-free RPMI medium for 6 h. The cells were further incubated with or without galectin-8 for the indicated times. At the end of incubation cells were washed once with PBS, and total RNA was isolated by homogenizing the cells that were grown in 100-mm tissue culture plates in TRI Reagent. The homogenates were incubated for 5 min at room temperature. Chloroform (1 ml) was then added, and the mixture was mixed vigorously and incubated at room temperature for 5 min. After centrifugation at 12,000 x g at 4 °C for 15 min, the aqueous phase was transferred into a fresh tube and 0.8 ml of isopropanol was added, followed by incubation at room temperature for 10 min. After centrifugation, the RNA pellet was washed once with 75% ethanol and dissolved in diethyl pyrocarbonate-treated water. RNA samples were translated to cDNA using a mixture of 10 mM dithiothreitol, 0.5 mM dNTP, 200 units of Moloney murine leukemia virus reverse transcriptase, 20 units of RNase inhibitor, and 250 ng of random hexamers. Samples were incubated at 25 °C for 10 min, 37 °C for 50 min, and 72 °C for 10 min. Quantitative PCR analysis in PRISM 7000 was performed using SYBR Green PCR master mix from Applied Biosystems International according to the manufacturer’s instructions. The reaction contained 50 ng of cDNA and 500 nM specific primers for human p21(5'-TGAGCCCGCA-CCTTGATG-3' (forward) and 5'-TCTCGGCTGACAAAGCTCGAAGTTCC-3' (reverse)). Results were normalized for the expression of human glyceraldehyde-3-phosphate dehydrogenase using 5'-ACCCACTCCTCCA-CCTTTGA-3' (forward) and 5'-CTGTTGTCTGTAGCAATTTGCTG-3' (reverse) primers.

**Northern Blot Analysis—**10-µg samples of total RNA were separated on 1% formaldehyde-agarose gel, transferred to Hybond-XL membrane, and hybridized with a [α-32P]dCTP-labeled p21 probe corresponding to the coding sequence of the human p21 gene (630 bp) (GenBank™ accession number BC013967). Membranes were exposed to x-ray film for 5 h at -20 °C. The blot was stripped and re-hybridized with β-actin probe under the same conditions for sample loading controls.

**RESULTS**

**Generation of Cells Overexpressing Galectin-8—**We have shown previously that transfection of H1299 cells (human nonsmall cell lung carcinoma) with galectin-8 cDNA significantly reduces (~75%) the number of G-418 resistant colonies when compared with cells transfected with an empty vector (24). Consistent with these findings, no stable colonies could be detected when naive CHO (CHO-P) cells were transfected with the gene encoding GFP-galectin-8 (not shown). To override the cytostatic effects induced by the overexpressed galectin-8, its
cDNA was introduced into CHO cells that overexpress the insulin receptor (CHO-T cells). These cells were chosen under the assumption that the growth-promoting signals provided by the overexpressed insulin receptors would overcome the growth-inhibitory effects of galectin-8. Overexpression of GFPGalectin-8 could be detected 24 h post-transfection by Western immunoblotting with galectin-8 antibodies (Fig. 1A, left). Cells overexpressing galectin-8 continued to accumulate up to 72 h post-transfection, but their number started to decline thereafter (Fig. 1B). The ability to overexpress galectin-8 by transient transfection prompted us to generate stable cell clones that overexpress this protein. The overexpressed GFP-galectin-8 maintained its biological activity as a lectin. This was evident by its ability to bind to a column of lactosyl-agarose (Fig. 1A, right). Furthermore, similar to its endogenous counterpart (24), the overexpressed galectin-8 was secreted and remained bound to the cell surface. Flow cytometry analysis for the presence of secreted cell surface GFP-galectin-8 revealed that binding of galectin-8-antibodies to the surface of intact CHO-GFP-gal-8 cells was ~3-fold higher than that of antibodies binding to CHO-GFP cells (Fig. 1C). Similar results were obtained in CHO cells that transiently overexpress Myc-tagged galectin-8 (CHO-Myc-gal-8, Fig. 1D). The secreted protein maintained, at least partially, its binding to cell surface receptors through protein-sugar interactions. This was evident by the fact that secreted galectin-8, introduced into CHO cells by transient transfection, could be partially dissociated from the cell surface upon incubation with lactose (Fig. 1E).

**Overexpression of GFPGalectin-8 Inhibits Cellular Proliferation**—To better characterize the effects of galectin-8 on cell growth, the fate of cells overexpressing galectin-8 was studied. CHO-T cells, which transiently overexpress GFPGalectin-8, exhibited reduced DNA synthesis as evident by their reduced ability to incorporate BrdUrd when compared with control (GFPG-transfected) cells (Fig. 2A). Similar results were obtained in CHO-T cells that stably overexpress GFPGalectin-8. BrdUrd incorporation was significantly reduced (~50%) in these cells, when compared with BrdUrd incorporation into control GFPG-transfected cells (Fig. 2B). The effects of the overexpressed galectin-8 could be mimicked by incubation of H1299 cells with soluble galectin-8. As shown in Fig. 2C, such treatment reduced by 50% the rate of [³H]thymidine incorporation into the cells. These findings suggest that overexpression of galectin-8 or the addition of soluble galectin-8 attenuates the rate of DNA synthesis, leading to the inhibition of cell growth.

**Galectin-8 Induces Elevation in the Cellular Content of the Cyclin-dependent Kinase Inhibitor p21**—The attenuated cell growth induced by galectin-8 could involve an increase in the cellular content of cyclin-dependent kinase inhibitors such as p21 (waf-1) (6). Indeed, elevated levels of p21 were already evident 4 h following the incubation of H1299 cells with galectin-8, and a maximal increase was observed by 24 h (Fig. 3A). The effects of galectin-8 were specific, because it did not increase the level of expression of other proteins like Bcl-2, Fas (not shown), PKB, or ERK1/2 (Fig. 5). The effects of galectin-8 were induced upon galectin-8 binding to cell surface glycoconjugates, because soluble galectin-8 failed to increase the cellular content of p21 when the cells were incubated in the presence of TDG, which inhibits protein-sugar interactions (Fig. 3B).

To determine the mechanism by which galectin-8 increases the cellular content of p21, changes in mRNA levels of the p21 gene were monitored by quantitative reverse transcription PCR. As shown in Fig. 4A, elevated levels of p21 mRNA (3–6 fold) were detected in H1299 cells treated with galectin-8 for as short as 3 h, and they remained elevated for at least 18 h. The change in the mRNA level of p21 was also assayed by Northern blot, and results similar to those obtained by quantitative reverse transcription PCR were obtained (Fig. 4B). No increase in the cellular content of p21 was observed when 1299 cells were incubated with galectin-8 in the presence of cycloheximide, an inhibitor of protein synthesis (Fig. 3B), indicating that the effects of galectin-8 could not be solely attributed to the inhibition of p21 degradation.

The specificity of the effects of galectin-8 were evaluated next. As shown in Fig. 3C, galectin-8 also induced the accumulation of p21 in PC3, CWR22RV1, and Saos-2 cells. Maximal
effects of the lectin varied in the different cells from 6 h in Saos-2 cells to 24 h in the H1299, PC3, and 22RV1 cell lines. Still, certain cell types resisted the accumulation of p21 induced by this lectin. As shown in Fig. 4C, galectin-8 failed to increase p21 mRNA levels in primary human foreskin fibroblasts, indicating that galectin-8 enhances the expression of p21 only in selected cell types.

**Induction of Growth Arrest by Galectin-8 Is Associated with Activation of JNK and PKB**—The stress-activated JNK and p38 MAPK (37), as well as PKB, were implicated in the elevation of the cellular content of p21 by two distinct mechanisms. Whereas JNK and p38 promote transcription of the p21 gene (38, 39), PKB phosphorylates and inhibits the activity of GSK3β that phosphorylates p21 and triggers its degradation (40, 41). Because JNK, p38, and PKB are all activated upon integrin ligation (42, 43), and because galectin-8 triggers the signaling of integrins (25, 26), the effects of soluble galectin-8 on JNK, p38 MAPK, and PKB activities were studied. As shown in Fig. 5A, galectin-8-stimulated JNK activity in a dose-dependent manner. Maximal stimulation occurred following incubation with 5 μM galectin-8, and similar doses were required to fully induce the accumulation of p21. Stimulation of p21 and JNK required prolonged incubations with the lectin. As shown in Fig. 5B, significant accumulation of p-JNK was observed at 3 h, and elevated levels of p-JNK were still evident even 9 h following the addition of galectin-8. The increase in p-JNK preceded the increase in cellular content of p21 (Fig. 5B), in accordance with the notion that activation of JNK induces p21 accumulation (38). Induction of JNK was inhibited upon the inclusion of 10 mM TDG (Fig. 5C), indicating that this process, like the induction of p21, was mediated by protein-sugar interactions. Accumulation of p-JNK could not be attributed to the increase in its cellular content (Fig. 5B). This conclusion is further supported by the fact that galectin-8 effectively increased the cellular content of p-JNK even in the presence of cycloheximide. In contrast, prolonged incubation of H1299 cells with soluble galectin-8 failed to stimulate ERK (Fig. 5B) or p38 MAPK activity (not shown). These results suggest that soluble galectin-8 stimulates only selected members of the MAPK family.

We have shown previously that cell adhesion to immobilized galectin-8 results in robust and sustained activation of PKB (26). To determine whether activation of PKB could also account for the accumulation of p21, PKB activation in response to soluble galectin-8 was studied. As shown in Fig. 5A, soluble galectin-8-stimulated PKB activity in a dose-dependent manner, with maximal stimulation being obtained with 5 μM galectin-8. Like JNK, stimulation of PKB preceded the induction of p21 and was already maximal following 3 h of incubation with soluble galectin-8. (Fig. 5B). Hence, soluble galectin-8, like its immobilized counterpart, induced PKB activity that was followed by the accumulation of p21.

**Inhibitors of JNK and PI3K or Overexpression of a Kinase-inactive Mutant of SEK Abolishes the Increase in the Cellular Content of p21 Induced by Galectin-8**—Specific inhibitors were employed to establish the causal link between the activation of PKB and JNK and the increase in the cellular content of p21 induced by galectin-8. As shown in Fig. 6A, the induction of p21 mediated by galectin-8 was partially inhibited, whereas PKB activation was completely abolished when H1299 cells were treated with wortmannin, a selective inhibitor of PI3K, the upstream activator of PKB. Similarly, the JNK inhibitor SP600125 at 10–50 μM effectively inhibited both JNK activation and the accumulation of p21 (Fig. 6B), whereas an inhibitor of p38 MAPK (SB203580) failed to impair p21 accumula-
tion induced by galectin-8 (not shown).

To further establish the causal link between the activation of JNK by galectin-8 and the accumulation of p21, we made use of a kinase-inactive mutant of SEK1 (MAPK kinase 4), a dual-specificity kinase that activates JNK by phosphorylating it on Thr and Tyr residues (44). As shown in Fig. 7, the introduction
into H1299 cells of an HA-tagged kinase-inactive mutant of SEK1 in which Lys-129 at the kinase-active site was replaced by Arg (HA-SEKKR) significantly inhibited (by 50%) the ability of galectin-8 to promote the activation of JNK. This was accompanied by a drastic reduction (75%) in the capacity of galectin-8 to induce the accumulation of p21 in the cells that overexpress the SEK1 mutant. These results establish a causal link between JNK activation and p21 accumulation in galectin-8-treated cells, implicating SEK1 as an upstream activator of JNK along the galectin-8 signaling pathway.

**Reduced Cellular Content of p21 Potentiates the Ability of Galectin-8 to Induce Apoptosis—** Increased levels of p21 coupled with the induction of growth arrest is one of the means utilized by cells under stress to avoid an apoptotic process (41, 45). We have shown that soluble galectin-8 potentiates apoptosis of cells maintained in serum-free medium (24). Therefore, it was of interest to determine whether conditions that inhibit p21 accumulation support an apoptotic process induced by the soluble lectin. As shown in Fig. 8A, galectin-8 effectively induced apoptosis in a number of cell lines, exemplified by enhanced cleavage of PARP, a known marker of an apoptotic process (3). Inclusion of cycloheximide abrogated the accumulation of p21 as it sensitized the cells to the pro-apoptotic effects of galectin-8, evident by the enhanced PARP cleavage (Fig. 8B). The induction of PARP cleavage was inhibited upon the inclusion of 10 mM TDG (not shown), indicating that this process, like the induction of JNK and p21, was mediated by protein-sugar interactions between galectin-8 and cell surface glycoconjugates.

To establish the causal link between the reduction in p21 content and the increased rate of apoptosis induced by galectin-8, its effects were studied in HTC cells derived from p21-null mice (HTCp21−/− cells) (32, 33). As shown in Fig. 8C, the ability of galectin-8 to induce apoptosis almost doubled in the HTCp21−/− cells when compared with control naïve HTC cells. These findings support our model that p21 accumulation, induced by galectin-8, attenuates the apoptotic process induced by this lectin.

**DISCUSSION**

The present study provides evidence that galectin-8 inhibits cellular growth by promoting the accumulation of the cyclin-dependent kinase inhibitor p21. The accumulation of p21 is mediated, at least in part, through activation of JNK and PKB,
the phosphorylation of which is markedly increased in galectin-8-treated cells. When cells treated with galectin-8 fail to accumulate p21, they are subjected to an accelerated apoptotic process that involves PARP cleavage. The cytostatic effects of galectin-8 are antagonized by growth factors such as insulin, whose receptors, when overexpressed, enable cells to accommodate high concentrations of galectin-8 without undergoing apoptosis. These findings implicate galectin-8 as a modulator of cell growth whose action is controlled by the availability of selected growth factors.

Several lines of evidence support such a model. First, failure to stably overexpress significant amounts of galectin-8 in H1299, CHO, NIH 3T3, or HEK 293 cells, which otherwise readily overexpress a variety of other proteins, indicates that the overexpressed lectin exerts growth-inhibitory effects in the transfected cells. This is evident by the reduced BrdUrd incorporation into CHO cells transfected with a GFP-galectin-8 and the reduced rate of DNA synthesis of H1299 cells treated with soluble galectin-8. These results are consistent with our previous findings demonstrating that overexpression of galectin-8 cDNA in H1299 cells results in a significant (75%) reduction in the number of G418-resistant clones (24). Galectin-8 can be overexpressed once cellular growth is promoted by the overexpression of receptors for a growth factor such as the insulin receptor, indicating that the cytostatic effects induced by galectin-8 are overridden by signaling pathways that trigger cellular growth. The overexpressed galectin-8 is biologically active. It retains its sugar binding activity and, like its endogenous counterpart (24), is secreted, remains associated with the cell surface, and promotes integrin signaling.

The underlying cause of growth arrest induced by galectin-8 is its ability to promote the accumulation of the cyclin-dependent kinase inhibitor p21. Initially considered only as an inhibitor of cell proliferation, increasing evidence now suggests that p21 confers apoptosis protection (41, 45) and might be important for cell survival (40, 46). Accordingly, a complex mechanism regulates the cellular content of p21. In addition to transcriptional induction by p53-dependent and p53-independent mechanisms, both ubiquitin-mediated as well as ubiquitin-independent degradation processes regulate the levels of p21 (reviewed in Ref. 47). In the present study we provide evidence that galectin-8, through the activation of JNK and PKB, increases the rate of synthesis of p21 and inhibits its degradation, a process that is already evident 3 h after the incubation of H1299 cells with galectin-8. Of note, the effects of galectin-8 on p21 accumulation were observed only in selected cell lines, whereas galectin-8 failed to exert a similar effect in human foreskin fibroblast cells. These findings suggest that the cytostatic effects of galectin-8 might be exerted mainly on transformed cells, because it fails to inhibit the growth of primary cultured cells.

Galectin-8 promotes the transcription of p21 mRNA by activating JNK that induces the activity of NF-κB, leading to p21 gene transcription (38). Accordingly, activation of JNK in response to galectin-8 precedes the increase in cellular content of p21. JNK can be activated by other galectins as well. JNK mRNA is increased when T-cells are incubated with galectin-1 (48) or when galectin-7 is overexpressed in HeLa and DLD-1 cells (49).

A causal link between the activation of JNK and the induction of p21 is provided by the fact that SP600125, a selective inhibitor of JNK, effectively inhibits p21 accumulation induced by galectin-8. Furthermore, introduction into H1299 cells of a dominant inhibitory mutant (K129R) of SEK1 (MAPK kinase 4), a dual-specificity kinase that activates JNK by phosphorylating it on Thr and Tyr residues (44), inhibits the ability of galectin-8 to activate JNK and to induce the accumulation of p21. The second signaling pathway utilized by galectin-8 to induce the accumulation of p21 is PKB. Soluble galectin-8 effectively stimulates PKB that promotes the accumulation of p21 by phosphorylating glycogen synthase kinase 3β (GSK3β). Such phosphorylation inhibits the activity of GSK3β that can otherwise phosphorylate p21 and tag it for proteosomal-mediated degradation (41). This effect of PKB may compensate for and even prevail over the destabilizing effect of PKB on p21-PCNA complexes (50) associated with the direct phosphorylation of p21 by PKB in vivo (51). Support for the role of PKB in the induction of p21 is provided by the fact that wortmannin, a selective inhibitor of PI3K, the upstream regulator of PKB, partially blocks the accumulation of p21 induced by galectin-8.

We have shown previously that galectin-8 serves as a ligand to a selected subgroup of integrins through protein-sugar interactions (24). Furthermore, both JNK and PKB are downstream effectors of integrins (52), some of which, like αvβ3 and αvβ5, induce the expression of p21 (53, 54). These observations set a link between integrin ligation by galectin-8, activation of PKB and JNK, and the cellular accumulation of p21. Still, JNK, rather than PKB, seems to play the major role in the mechanism underlying the accumulation of p21. This is evident by the fact that the inhibition of JNK activity completely inhibits p21 accumulation, even when PKB is still active. This finding suggests that the attenuated rate of degradation of p21 induced by PKB is of a lesser impact. Of note, the effects of galectin-8 on p21 accumulation must occur in a p53-independent manner, because H1299 cells lack a functional p53 (55). Other galectins also induce growth arrest. Galectin-1 functions as a cytostatic factor for murine embryo fibroblasts (12, 56) and galectin-3 induces cell cycle arrest of human breast epithelial cells (14), whereas galectin-12 inhibits growth of HeLa cells (13). Galectin-3-mediated G1 arrest involves down-regulation of cyclin E and cyclin A and up-regulation of p21 and p27 (14, 57), suggesting that both galectin-8 and galectin-3 induce growth arrest by a mechanism that involves the accumulation of p21.

Why does immobilized galectin-8 serve as an extracellular matrix protein that promotes cell adhesion, spreading, and growth (25, 26), whereas the soluble or the overexpressed lectin acts as a cytostatic factor? The opposing effects of galectin-8 could be attributed, for example, to the different concentrations of the lectin experienced by the cells. When galectin-8 is present at low concentrations as an immobilized ligand, it interacts only with the high affinity receptors of the integrin family (24) that promote cell migration and growth. In contrast, when galectin-8 is present at high enough concentrations as a soluble ligand or when it is overexpressed, it can interact with low affinity receptors that trigger its cytostatic effects. These receptors could either be other members of the integrin family or different cell surface receptors altogether. Support for this model is provided by the fact that the binding of galectin-8 to low and high affinity receptors results in a different repertoire of signals emitted when cells interact with immobilized versus soluble galectin-8. When cells adhere to immobilized galectin-8 and only the high affinity receptors are engaged, it triggers robust and sustained activation of the PI3K and MAPK pathways (25, 26). In contrast, when applied at high enough doses as a soluble ligand, galectin-8 triggers, in addition to PKB, a delayed response that involves the activation of stress-activated kinases like JNK, the expression of p21, and the induction of cytostatic effects. Of note, we and others have shown previously that the concentration of galectin-8 in certain tissues such as prostate cancer cells (58) or rat liver (22) is rather high. For example, in rat liver galectin-8 comprises 0.025% of
total Triton-extractable liver proteins. This suggests, although it does not prove, that galectin-8, being a secreted protein, might accumulate in relatively large amounts to affect the growth behavior of selected cell types.

The accumulation of p21 induced by soluble galectin-8 protects the cells from the potential pro-apoptotic effects of this lectin. This is evident by the fact that galectin-8 effectively potentiates apoptosis when serum-deprived cells are incubated with galectin-8 in the presence of inhibitors of protein synthesis that prevent the accumulation of p21. Furthermore, we could show that the ability of galectin-8 to induce apoptosis almost doubles in HTCo(2−/−) cells, which lack p21. Hence, the ability of galectin-8 to drive cells to apoptosis is masked by its ability to induce p21, which diverts the cells from the apoptotic pathway into growth arrest. The induction of growth arrest as a means to escape apoptotic process is well established; nevertheless, the apoptotic machinery has a complicated relationship to cell cycle control. Up-regulation of p21 and its binding to cyclin-dependent kinases may trigger growth arrest on the one hand, whereas the binding of p21 to caspase-3 may inhibit the function of the latter and inhibit pro-apoptotic processes (59). Similarly, the accumulation of cytosolic p21, which occurs during cellular differentiation (60), triggers the formation of complexes between p21 and the apoptosis signal-regulating kinase 1 (ASK1) (61). This results in the inhibition of ASK1 activity, which is otherwise required for the induction of programmed cell death (60). The mechanism by which galectin-8 promotes apoptosis presumably involves the activation of caspase 9 and caspase 3, which promote PARP cleavage. Still, other galectins implicated as inducers of apoptosis (62–65) seem to utilize somewhat different mechanisms. For example, apoptosis induced by galectin-1 involves a significant reduction in the cellular content of Bel-2 (64), whereas galectin-9 fails to affect the cellular content of this protein. Likewise, galectin-7 is associated with p53-dependent onset of apoptosis (65), whereas galectin-8 can induce apoptosis in a p53-independent manner. These observations indicate that different galectins can induce apoptotic processes by somewhat distinct mechanisms.

In summary, the present work, combined with previous studies (24–26), reveals that galectin-8 can act in three different modes, depending on the cellular context and the extracellular environment (Fig. 9). When it is immobilized in the presence of serum or selected growth factors, it interacts with high affinity receptors to promote cell adhesion, spreading, and cell migration (25). When galectin-8 is present at high concentrations as a soluble ligand or when it is overexpressed and secreted, it interacts with low affinity receptors that induce the accumulation of cyclin-dependent kinase inhibitors, represented by p21, that attenuate the rate of DNA synthesis and induce a cytostatic effect. The third mode, the pro-apoptotic mode of action of galectin-8, is exhibited either under conditions that prevent the accumulation of p21 or following a sustained deprivation of growth factors. These different modes of action of galectin-8 are mediated by different signaling pathways, with PI3K and MAPK being the predominant mediators of cell motility induced by immobilized galectin-8, JNK and p21 being key players in mediating the cytostatic effects, and JNK and caspases contributing to the pro-apoptotic effects of this lectin. In view of the cytostatic effects of galectin-8, it is no wonder that a number of tumor cells (66, 67), such as malignant colon tissues (68), attenuate the expression of this protein. Still, an interesting question is how galectin-8 is beneficial to invasive prostate carcinomas (58) and other tumors (69) that highly express this lectin. Further studies are therefore required to elucidate the cellular cues that dictate which mode of action of galectin-8 is operative under physiological or pathological conditions.

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