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Role of galectin-3 as a receptor for advanced glycosylation end products

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Role of galectin-3 as a receptor for advanced glycosylation end products. The advanced glycosylation end product (AGE)-binding proteins identified so far include the components of the AGE-receptor complex p60, p90 and galectin-3, receptor for advanced glycosylation end products (RAGE), and the macrophage scavenger receptor types I and II. Galectin-3 interacts with β-galactoside residues of several cell surface and matrix glycoproteins through the carbohydrate recognition domain and is also capable of peptide–peptide associations mediated by its *N*-terminus domain. These structural properties enable galectin-3 to exert multiple functions, including the modulation of cell adhesion, the control of cell cycle, and the mRNA splicing activity. Moreover, in macrophages, astrocytes, and endothelial cells, galectin-3 has been shown to exhibit a high-affinity binding for AGEs; the lack of a transmembrane anchor sequence or signal peptide suggests that it associates with other AGE-receptor components rather than playing an independent role as AGE-receptor. In tissues that are targets of diabetic vascular complications, such as the mesangium and the endothelium, galectin-3 is not expressed or only weakly expressed under basal conditions, at variance with *p90* and *p60* but becomes detectable with aging and is induced or up-regulated by the diabetic milieu, which only slightly affects the expression of *p90* or *p60*. This (over)expression of galectin-3 may in turn modulate AGE-receptor-mediated events by modifying the function of the AGE-receptor complex, which could play a role in the pathogenesis of target tissue injury. Up-regulated galectin-3 expression may also exert direct effects on tissue remodeling, independently of AGE ligands, by virtue of its adhesive and growth regulating properties.

A large body of experimental evidence indicates that the enhanced nonenzymatic glycation occurring in diabetes is implicated in the pathogenesis of long-term complications of the disease through several mechanisms [1]. Both early sugar adducts (Amadori products) [2] and advanced glycation end products (AGEs) [3] accumulate within tissues of humans and animals with diabetes and may induce injurious effects either directly [1] or through structurally distinct cell surface receptors [3, 4]; furthermore, both early and advanced glycation reactions are associated with oxidative stress [5, 6].

Among these mechanisms, the AGE/AGE-receptor-mediated pathway appears to play a pivotal role in modulating target tissue injury. In fact, this pathway is involved in both the removal of irreversibly glycated molecules, which is a major mechanism for protecting tissues from AGE-induced damage, and the activation of cell growth and secretory function, which results in a dysregulated process of tissue remodeling [3]. The altered remodeling triggered by AGE binding to cell surface receptors is characterized by enhanced deposition of extracellular matrix (ECM) [7, 8], as well as by altered cell growth and turnover [9]. These changes appear to be mediated by an abnormal pattern of expression of several cytokines, including the insulin-like growth factors [10], platelet-derived growth factor [11], transforming growth factor-β (TGF-β) [12], and vascular endothelial growth factor [13], through the activation of signaling molecules such as the p21(ras)-dependent mitogen-activated protein kinase (MAPK) [14] and its downstream targets and the transcription factors, NF-κB and the AP-1 complex [13, 14].

AGE-receptors are heterogeneous, just as their ligand AGEs. In fact, several AGE-binding proteins have been identified so far [15], including *p60*, a 50 kD protein homologous to a component of the oligosaccharyltransferase complex OST-48 (AGE-R1) [16]; *p90*, a 80 kD protein homologous to the PKC substrate 80K-H (AGE-R2) [16]; galectin-3, a 32 kD protein previously known as Mac-2 or carbohydrate-binding protein (CBP)-35 (AGE-R3) [17]; RAGE, a 35 kD member of the immunoglobulin

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Table 1. AGE-binding proteins

Protein	MW	Structural features	Function
p60 (AGE-R1)	50 kD	Homologous to the oligosaccharyl transferase complex OST-48 form a complex with DAD1 and ribophorin I and II in the endoplasmic reticulum	AGE uptake and degradation
p90 (AGE-R2)	80 kD	Homologous to the PKC and FGFR3 substrate 80K-H when phosphorylated binds the SH2 domain of Grb2 in a complex with Sos	cell activation
Galectin-3 (AGE-R3)	32 kD	Previously known as Mac-2 or carbohydrate-cell activation binding protein (CBP)-35 C-terminal CRD and N-terminal PGAY repeating domain	cell activation
RAGE	35 kD	Member of the Ig superfamily proteolytic fragment of a 45-kD protein forming complex with lactoferrin-like protein	cell activation oxidative stress
Scavenger receptor type I	220 kD	Homotrimeric protein with five domains: N-terminal cytoplasmic, transmembrane, spacer of 2-N linked sites, collagen-like triple helix, C-terminal cysteine-rich	AGE uptake and degradation
Scavenger receptor type II	170 kD	As scavenger receptor I except for the cysteine-rich domain, replaced by a 6-residue C-terminus	AGE uptake and degradation

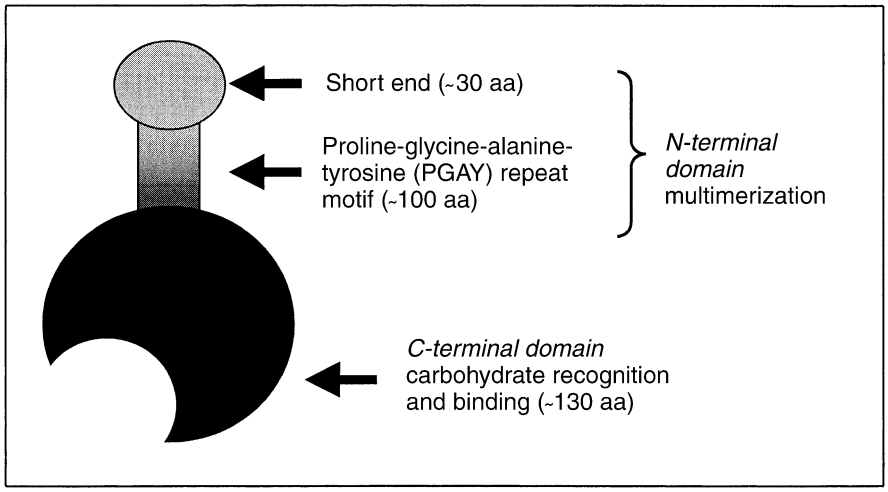

Fig. 1. Structure of galectin-3. (Modified from Barondes SH, Cooper DNW, Gitt MA, Leffler H: Galectins. Structure and function of a large family of animal lectins. *J Biol Chem* 269: 20807–20810, 1995)

superfamily of receptors [18]; and macrophage scavenger receptor type I and type II [19] (Table 1). Lactoferrin and lysozyme also exhibit heparin-and polyanion-inhibitable AGE-binding activity [20].

This paper focuses on the multifunctional molecule galectin-3, the most recently identified AGE-receptor, with special attention to its AGE-receptor function and relevance in the pathogenesis of long-term complications of diabetes.

GALECTIN-3 PROTEIN STRUCTURE AND GENE REGULATION

Galectin-3 belongs to the lectin family of adhesion molecules. Galectins are lactose/galactose-specific lectins (cation-independent) as opposed to selectins (cation-dependent) [21, 22]. Its structure consists of two domains, the C-terminal carbohydrate recognition domain (CRD), with highly conserved residues between members of the family, and the N-terminal domain, shared with galectin-4, with a unique short end continuing into an intervening proline-glycine-alanine-tyrosine-rich (PGAY) repeat motif (Fig. 1).

Galectin-3 interacts with the β-galactoside residues of several ECM and cell surface glycoproteins through the CRD. When bound to multivalent glycoconjugates, it is also capable of peptide-peptide homodimeric and heterodimeric associations [21, 22]. In the absence of its saccharide ligands, galectin-3 also self-associates in a C-terminus-dependent and carbohydrate-inhibitable manner [23, 24]. These structural properties enable galectin-3 to exert multiple functions that make it a broad-spectrum biologic response modifier [22].

In the human genome, galectin-3 is coded by a single gene, mapped to human chromosome 14 at region 14q21–22 [25]. It is composed of six exons and five introns, spanning a total of about 17 kb. Two transcription initiation sites are located upstream of the exon I–intron I border; the translation start site is located in exon II, the N-terminal domain within exon III, and the CRD within exon V [26].

Galectin-3 cell distribution and external secretion

Galectin-3 shows a ubiquitous localization within the cell, although it is localized mainly in the cytoplasm [27].

However, a significant amount of this lectin can also be detected in the nucleus, depending on the cell type and proliferative state. The phosphorylated form is found both in the nucleus of proliferating cells and cytoplasm of quiescent cells, whereas the nonphosphorylated form localizes exclusively in the nucleus of proliferating cells [28]. Moreover, serum-stimulated 3T3 fibroblasts show higher levels of galectin-3 than quiescent cells in culture [28, 29]. The expression (and localization) of galectin-3 also varies according to the replicative capacity of the cell, decreasing with aging in association with loss of galectin-3 regulation. In fact, younger cells respond to serum with a marked stimulation in galectin-3 expression and an elevation in the levels of the nonphosphorylated and phosphorylated protein, whereas older cells show high basal levels and respond to serum with a reduced protein and mRNA expression of galectin-3 [30].

Galectin-3 is secreted from cells into the extracellular space, although it lacks a signal sequence for transfer into the endoplasmic reticulum and Golgi compartments and entry into classical secretory pathways. In fact, it is externalized by a signal peptide and endoplasmic reticulum-Golgi complex independent mechanism, which requires the N-terminal half of galectin-3 [31].

Galectin-3 mRNA splicing activity and cell-cycle regulating function

Intracellularly, galectin-3 acts as a pre-mRNA splicing factor and also participates in the regulation of cell proliferation and death.

The mRNA splicing activity of galectin-3 has been postulated on the basis that most of this lectin is found in the form of ribonuclease A–sensitive ribonucleoprotein (RNP) complexes and that the N-terminal domain has limited similarities with proteins of the heterogeneous nuclear RNP complexes. In cell-free-splicing assays, nuclear extracts containing galectin-3 are capable of carrying out pre-mRNA splicing activity, which is inhibited by saccharides that bind galectin-3, but not by those that do not bind galectin-3 [32]. Splicing activity is reconstituted by addition of galectin-3 but not of other lectins with either similar or different saccharide binding specificity [32]. Nuclear extracts also contain galectin-1, which participates in the mRNA splicing activity together with galectin-3. In fact, depletion of both galectins from the nuclear extract results in a complete loss of splicing activity, whereas depletion of either galectin-1 or galectin-3 fails to abolish all of the splicing activity, and the residual splicing activity remains saccharide inhibitable [33]. Either galectin-1 or galectin-3 alone is sufficient to reconstitute, at least partially, the splicing activity of nuclear extracts depleted of both galectins. The CRD of galectin-1 or galectin-3 is also sufficient to restore splicing activity to a galectin-depleted nuclear extract, though at a greater concentration than the full-length polypeptide [33]. The nuclear ligand for galectin-3 (and galectin-1) has not been identified yet. Within the nucleus, galectin-3 and galectin-1 colocalize in speckled structures with the Sm epitopes of the small nuclear ribonucleoprotein complexes (snRNPs) and the non-snRNP splicing factor SC35, thus indicating a functional redundancy of nuclear galectins in their splicing activity and partitioning in the nucleoplasm with other splicing factors [34]. Within the nucleus, galectin-3 is also associated with single-stranded DNA (ssDNA) and RNA, with the highest affinity for poly(A) ribonucleotide homopolymers. Binding to either ssDNA or RNA is not inhibited by lactose [35].

The cell-cycle regulating properties of galectin-3 include the control of both cell replication and death through several possible mechanisms. The relation of galectin-3 to cell proliferation is indicated by its up-regulation and altered pattern of distribution and phosphorylation reported in 3T3 fibroblasts after mitogenic stimulation and before the onset of the S-phase of the cell cycle [27–30]. Moreover, in the rat model of acute mesangial proliferative glomerulonephritis induced by a single injection of anti-Thy1.1 antibodies, galectin-3 has been detected in the repopulating mesangial cell mass, thus suggesting a role for this lectin in mesangial hypercellularity [36]. Direct evidence of the mitogenic potential of galectin-3 has been obtained in normal human lung fibroblasts, with DNA synthesis and cell proliferation being stimulated in a dose-dependent, lactose-inhibitable manner requiring galectin-3 dimerization [37]. In addition to regulating cell proliferation, galectin-3 favors cell survival, as shown by the ability of this lectin to protect against the cytotoxic effects of TGF-β in rat mesangial cells grown in serum-free media [36]. A dual control of cell cycle by galectin-3 has been shown in human leukemia T cells or Jurkat cells. Galectin-3 is not expressed in these cells under starvation, but is induced or up-regulated by factors capable of activating the cyclic adenosine 3′,5′ monophosphate–responsive element binding protein and nuclear factor-κB (NF-κB) transcriptional pathways [38]. When transfected with galectin-3 cDNA, cells grow more quickly under low serum conditions and lower death rates in response to anti-Fas antibody or staurosporine compared with control transfectants [38]. That galectin-3 in the cytosol associates with *Bcl-2*, located on the outer membrane of mitochondria, in a lactose-inhibitable manner through the CRD suggests a direct participation of galectin-3 in cell death suppression pathways [38]. Galectin-3 shares evolutionarily related sequence homologies with *Bcl-2*, involving both the N- and C-terminal domains. The latter homology shows that galectin-3 contains the highly conserved NWGR motif present in the BH1 domain of members of the *Bcl-2* family and that it participates in the galectin-3/*Bcl-2* association [39]. Galectin-1, which does not contain an NWGR motif, induces apoptosis similarly to other

Table 2. Galectin-3 ligands

Cell surface	Extracellular environment
IgE receptor	IgE
Carcinoembryonic antigen (CEA)	Laminin
Colon cancer mucin	Tenascin
CD66	Fibronectin
Bacterial lipopolysaccharide	Collagen IV
Lysosomal-membrane-associated glycoproteins (LAMPs) 1 and 2	gp90/Mac-2 binding protein (M2 BP)
Mac-1	AGEs
Mac-3	
Heavy chain of CD98	
L1	
Myelin-associated glycoprotein	

members of the *Bcl-2* family [38]. Galectin-3 also inhibits apoptosis induced by the loss of cell anchorage (anoikis), with this inhibition involving cell cycle arrest at an anoikis-insensitive point (late G1) through modulation of gene expression and activities of cell cycle regulators [40]. By virtue of its proproliferative [37, 38] and antiapoptotic [38] action, galectin-3 is considered as an immediate early gene possibly implicated in tumor growth, as shown by the abnormal expression of galectin-3 reported in several neoplasias [41–43].

GALECTIN-3 ADHESIVE FUNCTION

As an adhesion molecule, galectin-3 regulates cell-to-cell and cell-to-matrix interactions through its CRD (Table 2). In the lectin-mediated cell adhesion, protein–carbohydrate interaction is exploited, not just peptide motif recognition, with the sugar code significantly contributing to the specificity of cellular selection of binding partners by integrating with other recognition modes. Cell surface galectin-3 molecules are capable of mediating homotypic cell adhesion by serving as a cross-linking bridge between adjacent cells, bridging through attachment to a complementary serum glycoprotein(s), which is inhibited by lactose [44]. Moreover, galectin-3 (and galectin-1) down-regulate cell adhesion to laminin through its association with $\alpha 1\beta 1$-integrin receptors in a lactose-inhibitable manner, thus producing an anti-adhesive effect [45, 46]. However, under certain circumstances, galectin-3 may promote cell adhesion to laminin, as is the case with neutrophils [47]. Alterations of tumor cell interaction with the basement membrane glycoprotein laminin are consistent features of the invasive and metastatic phenotype. Qualitative and quantitative changes in the expression of cell surface laminin-binding proteins, including a reduction of galectin-3, have been correlated with the ability of cancer cells to cross basement membranes during the metastatic cascade, and such phenotypic modifications have been shown to be associated with tumor aggressiveness and poorer prognosis [48–51].

Galectin-3 has been shown to bind IgE and the IgE receptor and to induce mast cell (and basophil) activation [52], thus suggesting a role for this molecule in allergy. Eosinophils [53] and neutrophils [54] also bind IgE through galectin-3, which therefore participates in their IgE-dependent effector function. Binding of galectin-3 to neutrophils is mediated by the carcinoembryonic antigen-related glycoprotein CD66 [55], and it results in activation of NADPH-oxidase [56] and superoxide production [57].

Other cell surface galectin-3 ligands are bacterial lipopolysaccharide [58]; the lysosomal-membrane-associated glycoproteins (LAMP) 1 and 2, adhesive glycoproteins [59]; the Mac-1 antigen, corresponding to the α-subunit (CD11b) of the CD11b/CD18 integrin; the Mac-3 antigen, a macrophage differentiation antigen related immunochemically to LAMP-2 and the heavy chain of CD98, a 125 kD heterodimeric glycoprotein [60]; and the cell recognition molecules L1, the neural cell adhesion molecule, and the myelin-associated glycoprotein [61].

Other ECM proteins that are capable of binding to galectin-3 include tenascin [61], fibronectin, collagen IV [46], and gp90/Mac-2 binding protein (M2 BP). The last is a multidomain and heavily glycosylated 90–97 kD protein showing homology with the cysteine-rich SRCR domain of the scavenger receptor in domain 1 [62]. It is secreted by various cells and is present in normal serum as well as in the ECM in the form of linear and ring-shaped oligomers [63], which interact with galectin-3, fibronectin, collagen IV, collagen V, collagen VI (but not I and III), nidogen, and $\beta 1$ (but not $\alpha 2$ and $\alpha 6$) integrin subunits [64].

Interactions of galectin-3 with the ECM also include the cleavage of this lectin by two members of the matrix metalloproteinase (MMP) family of enzymes, MMP-2 and MMP-9, and the inhibition of hydrolysis by tissue inhibitor of metalloproteinase (TIMP)-2 [65]. The major cleavage site is at the Ala62-Tyr63 bond contained in the N-terminal domain of galectin-3, with its hydrolysis generating an approximately 9 kD polypeptide comprising the N-terminal end of the intact galectin-3 and a 22 kD fragment with intact CRD [65]. However, formation of the 22 kD fragment alters the CRD so that it binds more tightly to the glycoconjugates, thereby reducing self-association of the galectin molecules and abrogating the biologic properties dependent on such associations [66]. Finally, ECM turnover appears to be modulated by galectin-3, given that its addition to primary cultures of rat mesangial cells increases the synthesis of collagen IV and acts in synergy with TGF-β on matrix synthesis by producing a quantitatively similar stimulatory effect [36].

GALECTIN-3 AGE-RECEPTOR FUNCTION

The AGE-receptor function of galectin-3 was suspected on the basis of the isolation of a sequence corre-

sponding to galectin-3 by screening an expression library from activated macrophages with anti-*p90* antibody [17]. Among the members of the galectin family, galectin-3 (and to a lesser extent, galectin-4) has been found to exhibit high-affinity ^{125}I-AGE-bovine serum albumin (BSA) binding with saturable kinetics. This binding is fully blocked by excess unlabeled naturally formed AGE-BSA or synthetic 2-(2-furoyl)-4(5)-furanyl-1H-imidazole (FFI)-BSA, but it is not or weakly inhibited by either early intermediate glycation products or carbohydrate moieties specifically binding lectins, such as lactose. Scatchard plot analysis is consistent with a single class of binding sites and an affinity on the order of magnitude of the AGE-receptor and higher than that of carbohydrates, with binding activity retained by the C-terminal domain and even enhanced by removal of the N-terminal domain. In addition to *p90*, immunoprecipitation with anti-galectin-3 reveals galectin-3 as well as galectin-3-associated proteins (40 and 50 kD) displaying AGE-binding activity. Galectin-3 interacting with AGEs assumes a distinct patchy distribution on the cell surface, due to the formation of high-molecular weight complexes between galectin-3 and other membrane components, which seem to be significant for displaying its AGE-receptor function [17]. The capacity of galectin-3 to bind to AGE, which was first demonstrated in macrophages, has since been confirmed in other cell types. Galectin-3 from human astrocytes has been shown to bind AGE-modified proteins, with binding being blocked by the corresponding antibody [67]. Likewise, galectin-3 expressed in human umbilical vein endothelial cells has been reported to bind labeled AGE-BSA, but FFI-BSA fails to compete with AGE-BSA for binding, which differs from data in other cells [68].

The lack of a transmembrane anchor sequence or signal peptide suggests that galectin-3 is associated with other AGE-receptor components rather than playing an independent role as an AGE-receptor. The macrophage scavenger receptor could be one candidate, because of its homology with M2 BP [62], in addition to *p90*, which was originally coisolated with *p60* from liver membranes [69]. At present, *p60*/AGE-R1, *p90*/AGE-R2 and galectin-3/AGE-R3 are believed to act as a molecular complex (the so-called "AGE-receptor complex"), with the first being implicated mainly in AGE uptake and degradation and the latter two being involved predominantly in cell activation [15]. In fact, *p90*/AGE-R2 seems to have no or weak AGE-binding activity, because binding of AGE-modified proteins is inhibited by anti-80K-H antibodies in U87MG glioblastoma cells [16], but not in human astrocytes [67] and umbilical vein endothelial cells [68]. Conversely, it may be involved in signal transduction, as shown by its phosphorylation after exposure to AGE-BSA, due to its sequence similarity with the 80K-H protein [16]. This molecule, originally characterized as a PKC substrate [70], was later shown to be phosphorylated by stimulation of cells with acidic or basic fibroblast growth factor (FGF), followed by its translocation on the plasma membrane where it binds to the SH2 domain of adaptor protein, growth factor receptor-bound protein 2 (Grb2), in a complex with the guanine nucleotide releasing factor Sos [71]. One study has been shown that 80K-H is involved in the signal transduction linking FGF receptor 3 (FGFR3) to MAPK, with activated FGFR3 stimulating 80K-H phosphorylation and interacting with the phosphorylated protein in a complex with Grb2-Sos and another protein p66 [72]. That 80K-H participates in the activation of the p21(ras)-MAPK pathway suggests that *p90* can also participate in the signal transduction triggered by RAGE [14].

However, the molecular mechanisms by which AGE-binding proteins associate and cooperate with each other to specifically recognize the various AGE structures and mediate their effects remain largely unknown. In fact, inhibition of either *p60* or *p90* with blocking antibodies has been shown to result in complete blockade of AGE-induced up-regulation of ECM proteins and growth factors in cultured mesangial cells [10, 11], suggesting that both receptors are necessary in the modulation of dysregulated tissue remodeling. Moreover, a predominant involvement of galectin-3 in cell activation has recently been questioned by our report that the galectin-3 knockout mouse develops accelerated glomerulopathy after induction of diabetes compared with the corresponding wild type animal. In galectin-3 deficient mice, the more pronounced proteinuria and mesangial expansion are associated with more marked glomerular AGE accumulation compared with the galectin-3 expressing mice, despite comparable degrees of metabolic derangement [abstract; Pugliese et al, *Diabetologia* 41(Suppl):A29, 1998]. These data suggest that galectin-3 deficiency may cause reduced removal of irreversibly glycated molecules, resulting in enhanced AGE-mediated injury rather than decreased AGE-induced cell activation. In sharp contrast, a lack of other AGE-independent galectin-3 actions has not been found to contribute to the development of glomerulopathy [abstract; Pugliese et al, *Diabetologia* 41(Suppl):A29, 1998].

Galectin-3 expression in health and disease

Galectin-3 expression appears to be developmentally regulated, being more abundant during embryogenesis and development than in adult life. Both galectin-3 and galectin-1 are first expressed in the trophectoderm cells of the implanting embryo, thus suggesting a role for these lectins in implantation. However, the lack of galectin-1 in galectin-1 null mutant mice and the lack of both galectin-1 and galectin-3 in double mutant mice are compatible with implantation [73].

During embryogenesis, galectin-3 and galectin-1 are

differentially expressed in tissues, suggesting their participation in the complex process of tissue differentiation [74, 75]. In the first trimester, galectin-3 is expressed mainly in epithelia (skin, digestive and respiratory tracts, and urothelium and excretory tubes of the kidney) but also in myocardial cells, chondrocytes, notochord, and liver, whereas galectin-1 is expressed in connective and derived tissues, such as smooth and striated muscle cells, and in skin and gonadal epithelia [76]. However, the finding that animals homozygous for an allele carrying a null mutation in the galectin-3 gene develop normally and are viable and fertile suggests that other proteins compensate for the lack of galectin-3 [77].

Postnatally, galectin-3 is detected in several tissues, with its expression being altered either quantitatively or qualitatively in various disease states.

Cells of the myeloid lineage, including monocyte-macrophages [60], neutrophils [54], eosinophils [53], and particularly basophils and mast cells [78], express galectin-3 under normal conditions. As previously mentioned, galectin-3 is involved in the activation of these cells, with pathogenetic implications in diseases, such as allergy, characterized by participation of bone-marrow-derived elements. Conversely, in B and T lymphocytes, galectin-3 is not expressed, although it is induced by infection of T cell lines with human T-cell lymphotropic virus-I and human immunodeficiency virus through the *Tax* and *Tat* genes, respectively [79, 80], and in some types of lymphoma [81].

In bone, galectin-3 is expressed in cells of the osteoblastic and chondroblastic lineages during differentiation and is up-regulated in hypertrophic conditions [82]. Conversely, it is down-regulated during osteoclast formation from monocytes [83].

In the nervous system, galectin-3 has been demonstrated in certain neurons [84] and particularly in glial cells [67]. An up-regulated expression of galectin-3 has been reported in primary astrocytomas and metastatic tumors in the central nervous system [43]. Although in astrocytomas, galectin-3 content decreases from low- to high-grade tumors, some tumor cell clones expressing high amounts of galectin-3 emerge with increasing levels of malignancy and tumor invasiveness [85].

Epithelia are major sites of galectin-3 expression, particularly epidermal keratinocytes and epithelial cells of the upper respiratory tract, gastrointestinal mucosa, urinary tract, pancreatic ducts, breast, uterus, and prostate. A reduced expression of galectin-3 has been observed in several carcinomas, including epidermal [48], breast [49], and prostate [50]. Conflicting data have been reported regarding galectin-3 levels in colorectal cancer, with either increased expression [86] or reduced (nuclear) content [51] during progression from normal mucosa to adenoma to carcinoma and in liver metastasis. The renal tubule epithelial cell also expresses galectin-3, which is confined to the α-intercalated cell in the collecting duct of a normal kidney, but it exhibits the apical expression pattern typical of the mesonephric duct/ureteric bud lineage during embryogenesis in diseased epithelia of both multicystic dysplastic kidneys and autosomal recessive polycystic kidneys [87]. Unlike tubules, neither glomeruli nor cultured mesangial cells express galectin-3 [36, 88], which, however, becomes expressed both in vivo and in vitro with increasing age of animals and cells, respectively [88]. In young animals, galectin-3 expression is induced during experimental glomerulonephritis by anti-Thy1.1 antibodies, with the lectin initially detected in recruited macrophages and later in proliferating mesangial cells [36]. In liver, normal hepatocytes do not express the lectin, but it becomes detectable in the cirrhotic liver as well as in hepatocellular carcinoma, independently of earlier hepatitis B virus infection [42]. In normal thyroid cells and in most benign thyroid lesions, galectin-3 is not expressed, whereas it is detected in thyroid malignancies of follicular and, less frequently, parafollicular cell origin, thus representing a marker for preoperative identification of malignant thyrocytes on cytologic specimens obtained by fine-needle aspiration biopsy [41].

In the vessel wall, smooth muscle cells do not express galectin-3, whereas endothelial cells show low but detectable levels of this lectin. Interestingly, galectin-3 is induced in proliferating vascular smooth muscle cells as well as in cells (smooth muscle and foam cells in particular) from arteries of experimental animal models of atherogenesis and human patients with advanced atherosclerotic lesions [89, 90].

Galectin-3 in diabetes and its relevance to diabetic complications

As already reported, galectin-3 is present in tissues that are sites of long-term diabetic complications, including the nervous tissue, kidney, and vessels, although it shows differential expression among the cell types comprising these tissues.

In the tissues of the nervous system, galectin-3 is expressed in glial cells, with only a few neurons exhibiting staining for this lectin, at variance with *p60* and *p90*, which are expressed in neural cells. In cultured human astrocytes, the mRNA transcripts for AGE-R1, -R2, and -R3 do not appear to be regulated by AGEs, FFI, or phorbol ester [67].

Within the kidney, galectin-3 is expressed in a subset of tubular epithelial cells but not in glomerular cells, although it becomes expressed at this level with increasing age, both in vivo and in vitro. This age-related glomerular/mesangial expression of galectin-3 is accelerated and exacerbated by diabetes, in parallel with progressive ECM accumulation, but in the absence of significant proliferation. Tubular staining is also enhanced in diabe-

tes [88]. In contrast with galectin-3, *p90* is only slightly up-regulated, whereas *p60* is unchanged or reduced [abstract; He et al, *J Am Soc Nephrol* 7:1871, 1996]. In cultured mesangial cells, galectin-3 expression is induced by prolonged exposure to high glucose concentrations or on exposure to AGE-modified proteins [88].

In endothelial cells, components of the AGE-receptor complex are expressed under normal conditions and all of them, particularly galectin-3, are subjected to up-regulation in response to AGE-modified molecules [68]. This finding as well as the demonstration that galectin-3 is expressed in arteries of experimental animal models and human patients with atherosclerotic lesions [89, 90] also indicates a possible involvement of this lectin in the pathogenesis of diabetic (and nondiabetic) vascular disease.

Induction (or up-regulation) of galectin-3/AGE-R3 induced by diabetes and aging suggests that AGE accumulation occurring in these conditions is capable of modifying the expression pattern of the AGE-receptor complex. This view is supported by the finding that exposure of mesangial and endothelial cells to AGEs results in increased galectin-3 expression [68, 88]. The occurence of both qualitative and quantitative changes in the components of the AGE-receptor complex, with prevailing (over)expression of galectin-3, in tissues that are sites of long-term diabetic complications prompts speculation that these alterations participate in target tissue injury by interfering with the AGE-receptor function, thus modulating AGE-receptor-mediated events. The changes observed in the AGE-receptor complex might compensate for the increased AGE levels by increasing the tissue AGE-binding capacity alone or also by producing an impact on AGE-mediated cell activation, or by favoring transduction of AGE-derived signals possibly involving phosphorylation of *p90* [17]. However, the consequences of the altered expression of the AGE-receptor complex on the development of diabetic complications is difficult to establish, in light of the dual function of AGE-binding proteins as well as the incomplete knowledge of the mechanisms underlying galectin-3 receptor function and its interaction with other receptor molecules. The more pronounced AGE accumulation detected in the kidneys and glomeruli of galectin-3 knockout compared with wild type diabetic mice, despite similar degrees of metabolic derangement, suggests that galectin-3 deficiency impairs the removal of the enhanced amounts of AGEs formed during chronic hyperglycemia. As a consequence, up-regulation of this lectin occurring in diabetes might have a protective effect against AGE-mediated tissue injury.

Because of the multifunctional nature of galectin-3, overexpression of this lectin induced by diabetes may have further implications in the dysregulated tissue remodeling underlying diabetic vascular complications. Altered cell adhesion to the ECM may in fact participate in the accumulation of matrix components within the tissue. Up-regulation of galectin-3 may also favor expansion of the cell compartment by stimulating cell growth and proliferation and macrophage activation [36] as well as by producing an anti-apoptotic effect [38].

In conclusion, available data suggest that hyperglycemia is associated with an up-regulation of galectin-3, an AGE-binding protein and adhesive lectin, which might influence development of diabetic complications by modulating the AGE-receptor-mediated effects and possibly the tissue remodeling process.

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