Galectin 1 is involved in vascular smooth muscle cell proliferation

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Abstract

Objective: Smooth muscle cell (SMC) migration and proliferation are the key steps in the development of atherosclerosis and restenosis. Matricellular proteins have been implicated in cell adhesion, migration and proliferation. Here we investigated the role of the matricellular protein galectin-1 (Gal-1), a β-galactoside-binding lectin, in SMC proliferation in atheroma and DNA synthesis in cell culture.

Methods: Protein expression was visualised by tissue section immunostaining. RNA expression was analysed using Northern blot analysis. DNA synthesis of human vascular SMCs was determined by 3H-thymidine incorporation. Recombinant glutathione S-transferase–galectin-1 fusion protein (Gal FP) binding to extracellular matrix (ECM) proteins was measured by ELISA. Gal-1 binding to cells and ECM was estimated using 125I-labelled Gal FP.

Results: Prominent Gal-1 staining coincided with SMC proliferation in human coronary endarterectomy samples in organoid culture. In cell culture, Gal-1 mRNA was upregulated in growing SMCs. Gal FP increased serum-induced DNA synthesis of human SMCs on plastic or endogenous ECM, but not of a rat PAC1 SM cell line. Also, Gal FP slightly increased SMC adhesion to ECM. SMCs exhibited a complex pattern of receptor-ligand interactions with Gal FP. The Gal-1 binding to SMCs was much stronger than to ECM, produced by these SMCs. We identified new ECM proteins: thrombospondin, vitronectin and osteopontin, which bound to Gal FP in a dose- and β-galactoside-dependent manner in ELISA.

Conclusions: Gal-1 binding to SMCs was stronger than to ECM, although ECM of atherosclerotic blood vessels contained additional ECM proteins which bound to Gal-1. Gal-1 was upregulated during SMC growth and Gal FP enhanced serum-induced DNA synthesis in SMCs. Overall, Gal-1 upregulation is likely to provide a reinforcement of serum-induced events during vascular injury. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Vascular smooth muscle cells (SMCs) play a critical role in the normal and pathological responses of the arterial wall to injury. They are normally present in the arterial tunica media in the resting state. Vascular injury causes proliferation of these cells and their subsequent migration from media to intima, where they proliferate and deposit extracellular matrix (ECM). A combination of migration and proliferation of SMCs plays an important role in the pathogenesis of a number of cardiovascular diseases, including atherosclerosis and restenosis after angioplasty and coronary stenting [1,2].

An increasing number of studies have shown that cell adhesion is involved in the regulation of cell proliferation per se, and in cell proliferation stimulated by growth factors [3,4]. Normal non-transformed cells must adhere to ECM components in order to proliferate in vitro. This phenomenon is called anchorage-dependence for the progression of the cell cycle. The repertoire of integrins and composition of ECM determine whether a cell will survive, proliferate or exit the cell cycle and differentiate in response to soluble growth factors. A particular subset of integrins, α1β1 (collagen/laminin receptor), α5β1 (fibronectin receptor), and αvβ3 (broad specificity RGD receptor), were shown to link cell adhesion to the MAP kinase pathway and cell proliferation in response to mitogens in primary endothelial cells and several other cell types [5,6]. Further evidence of ECM involvement in cell proliferation came from studies showing that cell adhesion...
is necessary for the expression of cyclin D and A, and the activation of cyclinD–cdk4/6 and cyclinE–cdk2 complexes required for cell cycle progression from G0 to S phase [7–9].

Recently, a new class of matricellular proteins have been implicated in cell adhesion, migration and proliferation [10]. Matricellular proteins, such as thrombospondin (TSP), osteopontin (OSP), SPARC etc., interact with ECM proteins and cellular receptors, and thereby modify ECM-dependent cell responses. Recently, we showed that galectin-1 (Gal-1), a small β-galactoside-binding lectin, exhibited some properties of matricellular proteins. Gal-1 affected adhesion and migration of SMCs in cell culture by interacting with αβ1 integrins and ECM proteins laminin (LN), and cellular fibronectin (cFN) [11].

Gal-1 is a ubiquitous mammalian protein expressed in many tissues at low levels, but particularly abundant in skeletal, cardiac and smooth muscle [12–14]. Previously, this protein was shown to affect proliferation of non-vascular cell types [15–18]. The published data on the effect of Gal-1 on cell proliferation are contradictory. It inhibited growth of mouse embryo fibroblasts, but stimulated proliferation of the mouse fibroblast BALB3T3 cell line. Gal-1 also inhibited or stimulated cell growth of some types of tumour cells, depending on the Gal-1 concentrations. However, transformed and tumour cells have a deregulated cell cycle and very often lose anchorage-dependence of cell proliferation.

Here we investigated whether Gal-1 was involved in proliferation of human vascular SMCs in cell and organoid culture and analysed Gal FP binding to SMCs and to ECM proteins selectively expressed in atherosclerotic blood vessels. Our data suggested that Gal-1 was upregulated during serum-induced proliferation of SMCs and enhanced DNA synthesis in vascular human SMCs.

2. Materials and methods

2.1. Materials

The construction of the recombinant protein to fuse C-terminus of glutathione S-transferase (GST) and N-terminus of human galectin-1 was described in detail previously [11]. GST and GST–Gal-1 fusion protein (Gal FP) were purified as described previously [11]. Polyclonal anti-Gal-1 and anti-GST antibodies were described previously [11]. Laminin (LN) from basement membrane of Engelbreth–Holm–Swarm mouse sarcoma, human plasma and cellular fibronectin (pFN and cFN), human vitronectin (VN) were purchased from Sigma-Aldrich; human thrombospondin (TSP)– from Calbiochem-Novabiochem. Rat osteopontin (OSP) was kindly provided by SmithKline Beecham Pharmaceuticals. Monoclonal antibodies against the proliferating cell nuclear antigen (PCNA) were purchased from Sigma. Monoclonal antibody against macrophage CD68 antigen and SM α-actin were purchased from Dako.

2.2. Tissue preparation

Three coronary endarterectomy samples were obtained from patients undergoing coronary artery bypass graft surgery. The investigation conforms with the principles outlined in the Declaration of Helsinki (Cardiovascular Research 1997;35:2–3). Organoid culture was described previously [19,20]. Briefly, the tissue samples were cut into segments. A preculture control was fixed immediately, the other segments of tissue were cultured up to 10 days in RPMI 1640 (Gibco BRL) medium containing 30% foetal bovine serum (FBS), antibiotics and 2 mM of L-glutamine, then fixed in formol saline. Paraffin tissue sections were prepared and processed as usual, stained with rabbit antibody against Gal-1 and monoclonal antibodies against PCNA, SM α-actin and macrophage CD68 antigen, followed by secondary biotinylated antibodies, detected with AB complex / HRP (Dako) and 3,3′-diaminobenzidine as a substrate for HRP. Some sections were counter-stained in Mayer’s hematoxylin. The primary antibody was omitted from the negative controls.

2.3. Cell lines

The investigation conforms with the principles outlined in the Declaration of Helsinki. Human vascular smooth muscle cells originated from primary explant culture of saphenous vein fragments, which were obtained from patients undergoing coronary bypass grafting, and cultured in DMEM medium with 20% FBS as described previously [11]. SMCs of 3–6 passages were used in the study. SMC phenotype was verified by immunostaining with anti-SM α-actin antibody and by a characteristic hill-and-valley growth pattern. These cells were used in most experiments unless stated otherwise. Umbilical SMCs, isolated from human umbilical cord arteries and kindly provided by Dr N. Carragher (Yamanouchi Research Institute, Oxford), were cultured in the same way as venous SMCs. The rat PAC1 cell line derived from pulmonary arterial smooth muscle was cultured in DMEM medium with 10% FBS [21,22].

2.4. mRNA expression analysis

Total RNA was isolated from PAC1 cells and human SMCs using Trizol reagent (GIBCO BRL) according to manufacturer’s protocol. Proliferating cells were grown to 65–70% confluence in serum containing medium as described above.

The quiescent cells were prepared as follows: the cells were grown to confluence and synchronised in G0 phase by serum starvation in serum-free DMEM supplemented with insulin–transferrin–selenium (Gibco BRL) for 2 days,
followed by treatment with 5 ng/ml of TGFβ (R&D Systems), 10 ng/ml PDGF BB (Sigma) or 100 nM of angiotensin II (Sigma) in serum-free DMEM for 3 days. The medium was changed every day. Control quiescent cells were kept in serum-free DMEM for 5 days altogether. Northern blots with total RNA on Hybond N-membrane (Amersham) were prepared [23], hybridised to cDNA probes: human Gal-1 cDNA [11] and human glyceraldehyde 3-phosphate dehydrogenase (GAPDH) cDNA (kindly provided by Dr A.J. Woods, University of Leicester, UK), and stripped as described previously [24]. cDNA probes were labelled using Multiprime DNA labelling kit (Amersham). To estimate Gal-1 mRNA expression, we quantified the bands on the autoradiographs using image analysis (AlphaImager 2000, Alpha Innotech Corporation).

2.5. DNA synthesis

Briefly, 96 well plates were coated with either of GST or Gal FP (300 ng/well) by air-drying, and washed with PBS. Cells were grown to confluence, synchronised in G0 by serum starvation in serum-free DMEM supplemented with insulin–transferrin–selenium for 48 h, detached and plated on 96 well plates (5×10³ cells per well) in DMEM with 10% FBS and 1 μCi/well of [³H]-thymidine (ICN), and incubated for 24 h. They were then washed with PBS, dissolved in 1 M NaOH and counted.

To study the effect of Gal-1 on DNA synthesis on endogenous ECM, ECM was prepared as described previously [25]. Briefly, SMCs were grown and kept on 96 well plates for 7 days in FBS-containing DMEM; then cells were removed by consecutive washing of the plates on ice with hypotonic buffer containing 10 mM Tris–HCl, pH7.5, 0.1% BSA and 0.1 mM CaCl₂, followed by the same buffer with 0.25% NP40 and washing with PBS. ECM was incubated with 1% BSA or 300 ng/well of either GST or Gal FP in 1% BSA for 1 h at room temperature, washed with PBS and used for plating the cells as described above.

All data are shown as the means (% of control)±SD from at least three experiments conducted in eight wells for each variant. The 100% of radioactivity in controls ranged from 4×10³ to 13×10³ cpm in different experiments. All data are shown as the means (% of control)±SD from at least three experiments conducted in eight wells for each variant.

2.7. FACScan analysis

Detached live non-permeabilized SMCs (8×10⁵/ml) were incubated with 500 and 1000 ng/ml amounts of GST or Gal FP in PBS with 1% BSA for 30 min at 15°C (to restrict endocytosis), followed by incubation with rabbit anti-GST antibody and FITC-labelled anti-rabbit antibody (Dako), both in dilution 1:100, for 30 min at 15°C. The cell fluorescence was measured with a FACScan flow cytometer (Becton-Dickinson). The data were analysed on a Hewlett-Packard (HP9000) computer equipped with FACScan research software. Population gates were set using SMCs incubated with secondary antibody.

2.8. Gal FP binding assay

GST and Gal FP were labelled using the iodo-gen method: 100 μg of each protein were iodinated with 300 μCi of Na¹²⁵I (NEN Life Science Products) according to manufacturer’s protocol. The specific activity was adjusted to 1.75×10⁵ cpm/μg of proteins.

SMCs were grown to confluence in 10% FBS medium for 6 days on several identical 24 well plates. These plates were used for ECM- and SMC-binding studies. Cell number, averaged among 6 wells, was 3.5×10³ cells per well. To study Gal-1 binding to cells, SMCs were detached by trypsinisation and resuspended in DMEM, containing 1% BSA, followed by incubation with 0.25–12.5 pmol of Gal FP (10–500 ng) and GST in 500 μl of DMEM with 1% BSA for 1 h at 15°C to restrict endocytosis. After that, cells were washed with PBS, dissolved in 150 ml of RIPA buffer, containing 10 mM Tris–HCl, pH 7.0, 150 mM NaCl, 2 mM EDTA, 0.1% SDS, 1% NP40, 1% sodium deoxycholate, and counted.

To study Gal-1 binding to ECM, ECM was prepared as described above. ECM was blocked with 1% BSA in DMEM for 30 min on ice and incubated with 1.25–12.5 pmol of Gal FP and GST in 500 μl of DMEM that contained 1% BSA and protease inhibitors cocktail (Sigma) for 1 h at room temperature, washed with 1% BSA in DMEM. Bound radioactivity was extracted with 1 M NaOH and counted. To investigate β-galactoside dependent interactions, we carried out the binding assays in the presence of 100 mM lactose.

Gal-1 specific binding was determined as the difference between the values of binding of Gal FP and GST used in
the same molar concentrations. All data are presented as the means±SD of six samples. Scatchard analysis of Gal-1 binding to cells was performed by plotting the ratio of bound/free Gal-1 concentrations against bound Gal-1 concentration. As Gal-1 binding to ECM was low, we could not draw the Scatchard plot for ECM binding.

2.9. Gal FP binding to ECM proteins in vitro

ELISA assay was performed as described previously [11]. Briefly, 96 well plates coated with 300 ng/well of ECM proteins were incubated with indicated amounts of GST or Gal FP in PBS with 1% BSA, washed, incubated with rabbit antibody to GST and secondary HRP-linked anti-rabbit antibody, and processed as described previously. To investigate β-galactoside dependent interactions, the binding assays were carried out in the presence of 50 mM lactose. All data are shown as means of four ±SD.

2.10. Data analysis

Differences among the groups for the combined experiments were prepared using one-way ANOVA in Statistical Package for the Social Sciences, followed by Dunnett’s test to determine whether the treatment groups were different from the control group. P<0.05 was selected as the level of statistical significance. All quantitative data are presented as means±SD, with P values indicated in the figure legends.

3. Results

The SM origin of the cells in coronary endarterectomy samples was confirmed by immunostaining for SM α-actin. SMCs stained with antibody against SM α-actin were predominant in the media and intima of the tissue samples (Fig. 1). Macrophages, identified by immunostaining for CD68, were found in some, but not all examined sections. CD68-positive macrophages were observed as scattered cells close to the luminal surface (Fig. 1) or as aggregates in the lesions of atheromatous arteries (not shown). Gal-1 staining was not observed in preculture control samples (Fig. 1, Day 0). A progressive increase in Gal-1 staining was observed when coronary endarterectomy samples were cultured in the presence of serum for 4 (not shown), 7 (Fig. 1), and 10 days (not shown). The Gal-1 staining was observed mostly at a far distance from cell nuclei in the ECM of atherosclerotic tissue fragments. Limited areas of very strong Gal-1 staining were observed near the luminal surface of some samples. The progressive increase in Gal-1 staining coincided with increasing expression of PCNA protein (Fig. 1). Thus, Gal-1 was upregulated during SMC proliferation in coronary en-

arterectomy fragments exposed to serum in organoid culture.

To determine whether Gal-1 upregulation occurred at the RNA level and to identify the factors inducing Gal-1 expression, we studied expression of Gal mRNA in SMCs in cell culture using Northern blotting (Fig. 2). The levels of Gal-1 mRNA were increased 2–2.5 fold in proliferating cells in three examined SMC types. The treatment of SMCs with TGFB, PDGF and Ang II increased Gal-1 mRNA levels by 30–40% in human SMCs and the PAC1 cells. Hence, Gal-1 was upregulated at the mRNA and protein levels in growing SMCs exposed to serum in cell and organoid culture. For this reason we suggested that Gal-1 accumulation might have functional importance in SMC proliferation.

To investigate whether Gal-1 was involved in SMC proliferation, we studied DNA synthesis in SMCs in response to serum in the presence of Gal FP in cell culture. When SMCs were plated on Gal FP-covered plastic, DNA synthesis was increased up to 129% compared to DNA synthesis on plastic or GST-cover plastic (Fig. 3A). Also, an increase up to 120% was observed when SMCs were plated on endogenous ECM treated with Gal FP compared with ECM non-treated or treated with GST (Fig. 3B). The DNA synthesis was two and a half-fold higher on endogenous ECM than on plastic. Thus, Gal-1 increased DNA synthesis of human vascular SMCs on both plastic and endogenous ECM in the presence of serum. In a similar type of experiment, we studied whether Gal-1 would increase DNA synthesis of the SM cell line PAC1. Gal FP did not affect DNA synthesis in PAC1 SM cells (Fig. 3).

To study whether Gal FP has any mitogenic activity, we cultured SMCs in serum-free medium on Gal FP-covered plastic or ECM treated with Gal FP. Gal FP did not induce DNA synthesis (not shown). Since cell proliferation is influenced by cell adhesion, we investigated whether Gal FP would affect SMC adhesion to ECM (Fig. 4). SMC attachment was higher to ECM in all variants compared to plastic. Further treatment of ECM with Gal FP increased cell adhesion only slightly (108%), whereas treatment with GST did not have any effect.

Our previous data suggested that Gal-1 interacted with ECM proteins and cellular receptors, including LN and α1β1 integrin, in cell protein extracts [11]. Using flow cytometry, we detected a direct interaction between Gal FP and SMCs that indicated the presence of receptors for Gal-1 on the cell surface. The fluorescence of SMCs incubated with a mixture of anti-GST and secondary FITC-labelled antibodies in the absence or presence of GST was very similar, whereas the presence of Gal FP significantly increased SMC fluorescence (Fig. 5). The treatment of the cells with chondroitinase ABC did not abolish Gal-1–cell interactions (not shown). In order to estimate which of the Gal-1 interactions was more prominent, iodinated Gal FP and GST were used to study Gal-1 binding to SMCs and ECM produced by the same cells (Fig. 6). Gal-1 specific
Fig. 1. Gal-1 and PCNA immunostaining of sections of coronary endarterectomy samples. Top panels: the fragments of the same sample were fixed immediately (day 0) or fixed after 7 days in organoid culture and immunostained with antibodies against Gal-1 and PCNA (brownish colour). The cell nuclei were counter-stained in Mayer’s hematoxylin (violet colour). Bottom panel: immunostaining with antibodies against SM α-actin and CD68. Lumenal surface of the samples is uppermost. Bar, 20 μm.
binding was determined as a difference between binding of GST and Gal FP, used in the same molar concentrations. Surprisingly, Gal-1 binding to SMCs was 30–40-fold higher than binding to ECM produced by those cells (compare Fig. 6A and 6B). The Gal-1 specific binding to ECM was abolished by lactose, whereas Gal-1 binding to SMCs was stronger and reduced only by one-third in the presence of lactose. A Scatchard plot of cell binding showed a non-linear curve, which indicated either the presence of different types of receptor sites for Gal-1 or co-operative effects of ligand-receptor interactions (Fig. 6C).

Since we found Gal-1 in ECM of atheroma fragments, we studied the binding of Gal FP to the ECM proteins VN, OSP and TSP, which are known to be expressed by SMCs in atherosclerotic, but undetectable in normal blood vessels. The ELISA experiments showed that Gal FP bound to TSP and VN in a dose-dependent manner in the range 50–2500 ng (not shown). Gal FP also bound to OSP at lower levels (not shown). Since the values of the binding depend on the temperature and time of ELISA procedures, we compared Gal FP binding to several ECM proteins in parallel (Fig. 7). Gal FP binding to the same amounts of ECM proteins was dose-dependent and in the order: LN>cFN>TSP>pFN>VN>OSP. This binding was significantly reduced by lactose.

4. Discussion

It has been shown recently that Gal-3, a β-galactoside-binding 30 kDa lectin, is likely to be involved in the development of atherosclerosis, as it is expressed at increased levels in human atherosclerotic lesions, rat aorta after balloon injury and in hypercholesterolemic rabbits [26,27]. Here we present data on expression of another lectin, Gal-1, in human atheromatous endarterectomy samples exposed to serum. Upregulation of Gal-1 coincided with expression of PCNA protein, therefore the upregulation of Gal-1 expression coincided with SMC proliferation in the atheroma fragments of blood vessels.

There is little information on the expression of Gal-1 gene. It was shown that the Gal-1 promoter is regulated by the level of DNA methylation in several cell types, but Gal-1 expression in SM has not been studied [28]. Since we did not have sufficient amounts of tissue samples to isolate RNA, we studied Gal-1 upregulation in cell culture. Gal-1 mRNA levels were markedly increased in growing
SMCs exposed to serum compared to quiescent SMCs in human venous and arterial SMCs and in the rat PAC1 cell line. PDGF, TGFβ and Ang II affect expression of ECM proteins. PDGF and Ang II are also known to induce SMC proliferation. PDGF, TGFβ and Ang II slightly induced Gal-1 mRNA levels in quiescent venous SMCs and the PAC1 cells. Thus, we observed a significant increase in Gal-1 expression in proliferating SMCs exposed to serum in organoid and cell culture.

In an attempt to understand a potential function for Gal-1 in cell proliferation, we studied whether Gal-1 may affect cell growth in cell culture. DNA synthesis of vascular SMCs, induced by serum, was enhanced on Gal FP-covered plastic compared with non-covered or GST-covered plastic. Also, DNA synthesis on ECM produced by SMCs was increased when ECM was treated with Gal FP compared with non-treated or GST-treated ECM. The increase of DNA synthesis induced by Gal FP was higher on plastic than on ECM, possibly because ECM bound only a small proportion of the applied Gal FP (Fig. 6). Gal FP did not affect DNA synthesis of PAC1 cells. The PAC1 SM cell line, which is able to carry out SM-specific splicing, maintains a differentiated SM phenotype and expresses large amounts of SM α-actin [21,22], is an immortal cell line indicating that cell cycle control is deregulated. The loss of responsiveness to the ECM signals, e.g. the presence of Gal-1, may indicate the differences between normal SMCs and the immortal PAC1 cell line, and may relate to the growth requirements of normal vascular SMCs.

The Gal-1 effect on DNA synthesis of SMCs may be caused either by direct stimulation by Gal-1 or by increased adhesion mediated by Gal-1. Our data suggested that Gal FP did not have any mitogenic activity in serum free medium on plastic or ECM. As we showed previously, the effect of Gal FP on cell adhesion is quite complicated: although SMCs attached to Gal FP-covered plastic, Gal FP inhibited cell spreading, and thereby rearrangement of cytoskeleton required for cell proliferation. Gal FP slightly increased cell attachment to ECM. Since the increase in cell adhesion induced by Gal FP was much lower than the increase in DNA synthesis, it seems unlikely that Gal FP affects cell proliferation via increasing cell adhesion. We infer that Gal FP-induced modulation of cell adhesion and DNA synthesis may be independent events mediated via different receptors and signalling pathways.

Gal-1 has been shown to bind to ECM proteins as well as to cellular receptors [29–35]. The proteins recognised by Gal-1 are cell type specific. In carcinoma cells, the main cellular proteins bound to Gal-1 were identified as Lamp-1 and Lamp-2, and carcinoma embryonic antigen [33–35]. Gal-1 interacted with α7β1 and α1β1 integrins in skeletal myocytes and human SMCs, respectively [32,11].
in SMC extracts. Using flow cytometry, we detected direct interactions between SMCs and Gal FP, and the presence of Gal-1 receptors on the cell surface of SMCs. To estimate the main Gal-1 binding sites in SM, we compared the Gal-1 binding to SMCs and ECM produced by these cells in cell culture. Contrary to our expectations, the binding to SMC was 30–40-fold higher than to ECM. Scatchard plot of cell binding demonstrated the complexity of cell–Gal-1 interactions: either different types of cellular receptors were present on the cell surface or there was a co-operative effect of Gal-1–receptor binding. Gal-1 is known to have the highest affinity for acetyllactosamine-containing polysaccharide chains of glycoproteins and proteoglycans, but it also binds to other sugar structures with lower affinity [29,37]. Gal-1 exists in monomeric and dimeric forms. The dimer binds to carbohydrates with higher affinity than the monomer [37]. Crystallographic analysis revealed that Gal-1 dimers cross-link polysaccharide chains [38]. This cross-linking is thought to play an important role in Gal-1 involvement in different physiological phenomena [29,38]. Gal FP may not form dimers via the Gal-1 N-terminus sequences responsible for dimerisation, as these sequences may be hidden inside N-GST–Gal-1-C termini FP. However, the isolation procedure for GST FP is based on isolating active GST dimers which bind to substrate-linked beads [39,40]. For these reasons Gal FP is likely to form dimers, although not via Gal-1 interactions, which implies that Gal FP can cross-link polysaccharide chains as native Gal-1 dimers. Altogether, it is more likely that there are high and low affinity interactions between Gal FP and SMCs via several cell surface receptors.

Similarly, Gal-3, the other β-galactoside-binding lectin, was shown to associate with α1β1 integrin [36]. In placenta, the major Gal-1 binding proteins were laminin and fibronectin [31]. Laminin is the major Gal-1-binding protein in muscle cells and several other cell types [29,30].

We showed previously that Gal-1 inhibited SMC adhesion to LN by interacting with both cells and LN [11], which indicated interactions between SMCs and Gal-1. We also showed that Gal-FP bound to α1β1 integrins and LN.
Fig. 7. Gal FP binds to ECM proteins in ELISA. Gal FP and GST was used in ELISA to test Gal FP binding to osteopontin (OSP), vitronectin (VN), thrombospondin (TSP), cellular and plasma fibronectins (cFN and pFN) and laminin (LN). Horizontal axis: Gal FP and GST concentrations are shown in ng/ml, ECM proteins are shown below. Vertical axis: optical density readings (A 490 nm) were taken versus control without Gal FP, GST and ECM proteins. Gal FP is shown in diagonal stripes, Gal FP in presence of 50 mM of lactose is shown in dotted pattern, GST is shown in white.

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