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Involvement of galectin-1 in reproduction: past, present and future

Gabriela Barrientos[1,2], Nancy Freitag[1], Irene Tirado-González[1], Laura Unverdorben[3], Udo Jeschke[3], Victor L.J.L. Thijssen[4], and Sandra M. Blois[1,*]

[1]CharitéCenter 12 Internal Medicine and Dermatology, Reproductive Medicine Research Group, Universitätsmedizin Berlin, Berlin, Germany, [2]Laboratorio de Fisiología Molecular Placentaria, Instituto de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, CONICET, Buenos Aires, Argentina, [3]Department of Obstetrics and Gynaecology, Campus Innenstadt, Ludwig-Maximilians-University of Munich, Maistrasse 11, 80337 Munich, Germany and [4]Departments of Radiation Oncology and Medical Oncology, Angiogenesis Laboratory, VU University Medical Center, Amsterdam, The Netherlands

Correspondence address. Tel: +49-30-450-553791; E-mail: sandra.blois@charite.de

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BACKGROUND: After recognition of its pivotal contribution to fetomaternal tolerance, the study of galectin-1 (gal-1) functions in the context of pregnancy became an attractive topic in reproductive medicine. Despite considerable advances in the understanding of the immuno- and growth-regulatory properties of gal-1 at the fetal–maternal interface, many functional aspects of this lectin in reproduction are only emerging.

METHODS: The published literature was searched using Pubmed focusing on gal-1 signalling and functional properties at the maternal–fetal interface, including data on its implication in pregnancy disorders and malignancies of the female reproductive system. Papers discussing animal and human studies were included.

RESULTS: This review provides an overview of gal-1 functions during pregnancy, such as modulation of maternal immune responses and roles in embryo implantation and placentation. We also emphasize the role of gal-1 in key regulatory processes, including trophoblast migration, invasion, syncytium formation and expression of non-classical MHC class I molecules (HLA-G). In addition, we argue in favour of gal-1 pro-angiogenic properties, as observed in tumourigenesis and other pathological settings, and its implication in the angiogenesis process associated with early gestation.

CONCLUSION: The involvement of gal-1 in the regulation of different processes during the establishment, development and maintenance of pregnancy could be described as unique. Gal-1 has emerged as an important lectin with major functions in pregnancy.

Key words: galectin-1 / pregnancy / angiogenesis / progesterone / cancer

Introduction: the galectin family and galectin-1

Galectins constitute a phylogenetically conserved family of lectins characterized by their carbohydrate recognition domains (CRDs) sharing a consensus sequence of ~130 amino acids that confers the ability to bind β-galactoside-rich glycoconjugates (Barondes *et al.*, 1994). At least 13 of the 19 members of the family described to date are expressed in human tissues (Cooper, 2002), where they show a wide range of subcellular localizations and biological functions. Galectins lack a secretory signal sequence, but can still be found in the extracellular milieu after being exported from cells by a mechanism termed non-classical secretion (Nickel, 2005). There, they mediate functions dependent on their lectin activity, triggered by interaction of their CRDs with *N*-acetyllactosamine (LacNAc) residues common in many cell-surface and ECM glycoproteins (Ahmad *et al.*, 2004). In contrast, intracellular functions of galectins are due to their ability to engage in protein–protein interactions and include the modulation of cell growth, differentiation, survival and migration (Liu *et al.*, 2002).

The first member of this family was reported more than 30 years ago as a ± 15 kDa protein existing in a non-covalent homodimeric form which, depending on its source, was named electrolectin, β-galactoside-binding lectin, galaptin or L-14 (Cummings and Liu, 2009). After systematization of the nomenclature for galectins, the protein became known as galectin-1 (gal-1) encoded by the gene *LGALS1* (lectin, galactoside binding, soluble 1). The *LGALS1* locus maps to human chromosome 22q13 and consists of four exons, which generate a 0.6 kb transcript encoding a protein of 135 amino acids (Fig. 1). In terms of its structure, gal-1 is recognized as a 'prototypic' galectin consisting of a monomeric CRD with a typical 'jelly roll' folding structure composed of five- and six-stranded antiparallel β-sheets arranged in a β-sandwich (Barondes *et al.*, 1994, Cho and Cummings, 1995). At concentrations > 1–2 μM, human gal-1 can be found in solution as a dimer (Cho and Cummings, 1995; Lopez-Lucendo *et al.*, 2004; Leppanen *et al.*, 2005), which is maintained principally by non-covalent interactions involving the carboxy- and amino-terminal domains of each subunit leaving the glycan-binding sites in the CRD located at opposite ends (Lopez-Lucendo *et al.*, 2004) (Fig. 1). Being a member of the galectin family, the gal-1 CRD binds to beta-galactosides including lactose and LacNAc, but other ligands have also been reported, e.g. the carbohydrate portion of ganglioside GM1 (Kopitz *et al.*, 1998) and alpha-galactosides (Miller *et al.*, 2011). The affinity of these interactions depends on multiple parameters as elegantly shown by Leppanen *et al.* These authors combined monomeric or dimeric forms of gal-1 with a broad panel of glycans, either soluble or immobilized, and assessed binding affinity (Leppanen *et al.*, 2005). This showed that the interplay between monomeric and dimeric gal-1 forms and their subcellular localizations together with the presentation and structure of glycoligands allows for a great variety of biological functions ascribed to this lectin.

Pregnancy is a highly regulated event in which endocrine, immune and metabolic systems co-operate in order to sustain life. Although gal-1 expression in the female reproductive system was reported in the 1990s (Phillips *et al.*, 1996; Maquoi *et al.*, 1997), the functions of this lectin during pregnancy are only emerging. Given its proven influence on key processes involved in pregnancy maintenance, such as the modulation of angiogenic, immune responses and placental development, gal-1 is currently the subject of intensive research in the field of reproductive medicine. In this review, we provide an overview of the different functions of gal-1 in human and mouse reproduction. We describe its role as a tolerogenic, pro-angiogenic and growth-regulatory protein driving the maternal adaptations to pregnancy and we deliver insights into gal-1 signalling at the fetal–maternal interface. Finally, we discuss its implication in reproductive disorders including reproduction-related malignancies and its potential application for diagnosis and therapeutical interventions.

Methods

The published literature was searched using Pubmed focusing on gal-1 signalling and functional properties at the maternal–fetal interface, including data on its implication in pregnancy disorders and malignancies of the female reproductive system. Papers discussing animal and human studies were included.

Regulation of galectin-1 expression within the female reproductive system

Cyclic changes in preparation of the endometrium for implantation are regulated by the steroid hormones estrogen and progesterone, which mediate their function via the nuclear estrogen and progesterone receptors, respectively. Rising levels of estrogen during the first half of the cycle direct the proliferation of endometrial epithelial and stromal cells,

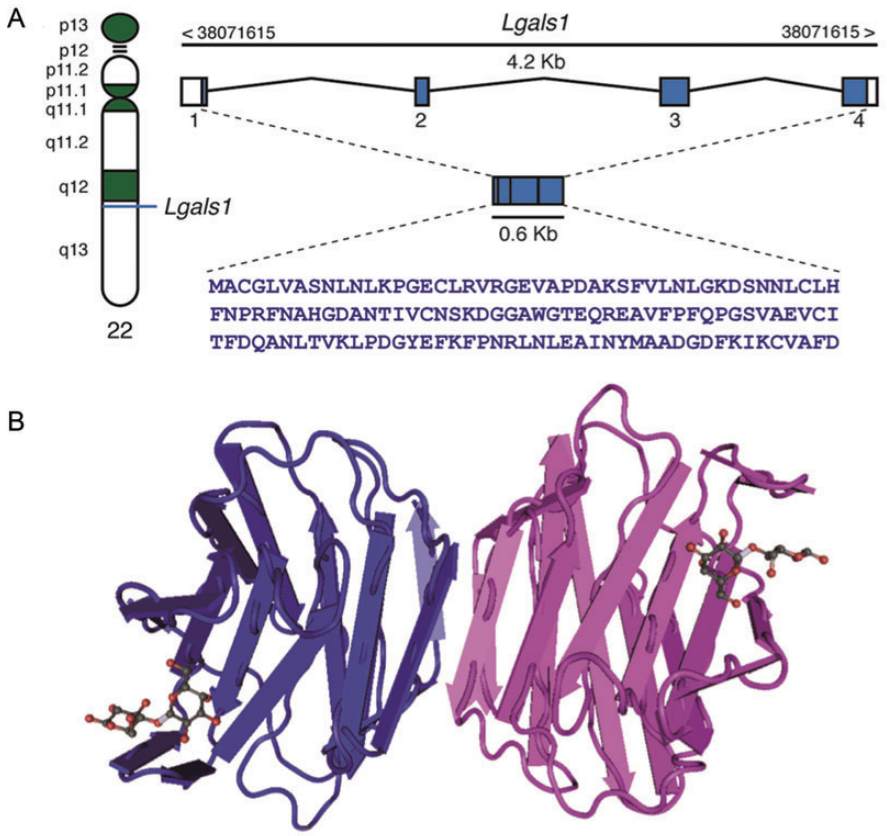

Figure 1 Structure of gal-1 at the gene and protein levels. (**A**) The *Lgals1* locus maps to human chromosome 22q12 and comprises ~4.2 kb. Splicing of four exons (boxes 1–4) gives rise to a 0.6 kb transcript encoding a protein of 135 aminoacids. (**B**) gal-1 is found in solution as a homodimeric form maintained by non-covalent interactions involving the N- and C- termini of its subunits (depicted in blue and pink). Each monomer presents the typical *jelly roll* folding structure of the CRD composed of five- and six-stranded antiparallel β-sheets arranged in a β-sandwich. The CRDs are illustrated in the figure as thelactose (Galβ1–4Glc) binding sites in each monomer. CRD, carbohydrate recognition domain.

whereas progesterone promotes the differentiation of the stroma playing a key role in defining the window of uterine receptivity during the secretory phase (Fig. 2).

Endometrial gal-1 expression fluctuates during the female cycle and gestation concomitant with regulation by steroid hormones (Choe *et al.*, 1997). Indeed, detailed investigations of the *LGALS1* promoter have revealed a half-estrogen responsive element and other elements that are induced by estrogen, e.g. nuclear transcription factor (NF)-Y and activator protein-2 (Orso *et al.*, 2004; Scafoglio *et al.*, 2006; Than *et al.*, 2008a, b). Interestingly, the *LGALS1* gene gained collectively 10 of these *cis* elements in the evolutionary tree of placental mammals, indicating that these functional changes may have been important in the emergence of maternal–fetal immune tolerance and are thus conserved in eutherian species (Than *et al.*, 2008a, b). In line with this, progesterone and estrogen were shown to raise gal-1 levels, while the blocking of their nuclear receptors with specific inhibitors abrogated this effect (Choe *et al.*, 1997). Additionally, mice deficient in progesterone or the immunophilin FK506-binding protein 52 (FKBP52), a protein that optimizes progesterone receptor function, display reduced levels of gal-1 expression (Hirota *et al.*, 2012). In delayed implantation experiments, estrogen together with progesterone, but not progesterone alone increased uterine gal-1 expression (Choe *et al.*, 1997), indicating that steroid hormone regulation of gal-1 may be particularly important during the phase of uterine receptivity in mice (Fig. 3).

In addition, we have shown that a functional cross talk between gal-1 and progesterone is important for the establishment and maintenance of immune tolerance during pregnancy. Thus, *in vivo* experiments demonstrated that gal-1 increases circulating levels of progesterone and progesterone-induced blocking factor (PIBF) and reciprocally, gal-1 expression on stromal and decidual cells is up-regulated after supplementary administration of dydrogesterone, a progesterone derivative, in a model of spontaneous abortion in mice (Blois *et al.*, 2007). Interestingly, *in vitro* studies show an inhibitory effect of gal-1 on progesterone secretion by ovarian granulosa cells as a result of interference with the FSH-receptor interaction (Walzel *et al.*, 2004). Furthermore, a sharp increase in gal-1 expression is detected in regressing corpora lutea of mice, followed by up-regulated levels of the progesterone-degrading enzyme 20α-hydroxysteroid dehydrogenase (20α-HSD) (Nio and Iwanaga, 2007). These findings, together with the almost absent expression of gal-1 in the corpora lutea of pregnancy (Nio-Kobayashi and Iwanaga, 2010), strongly implicate this lectin as a negative modulator of progesterone production and a signal of functional

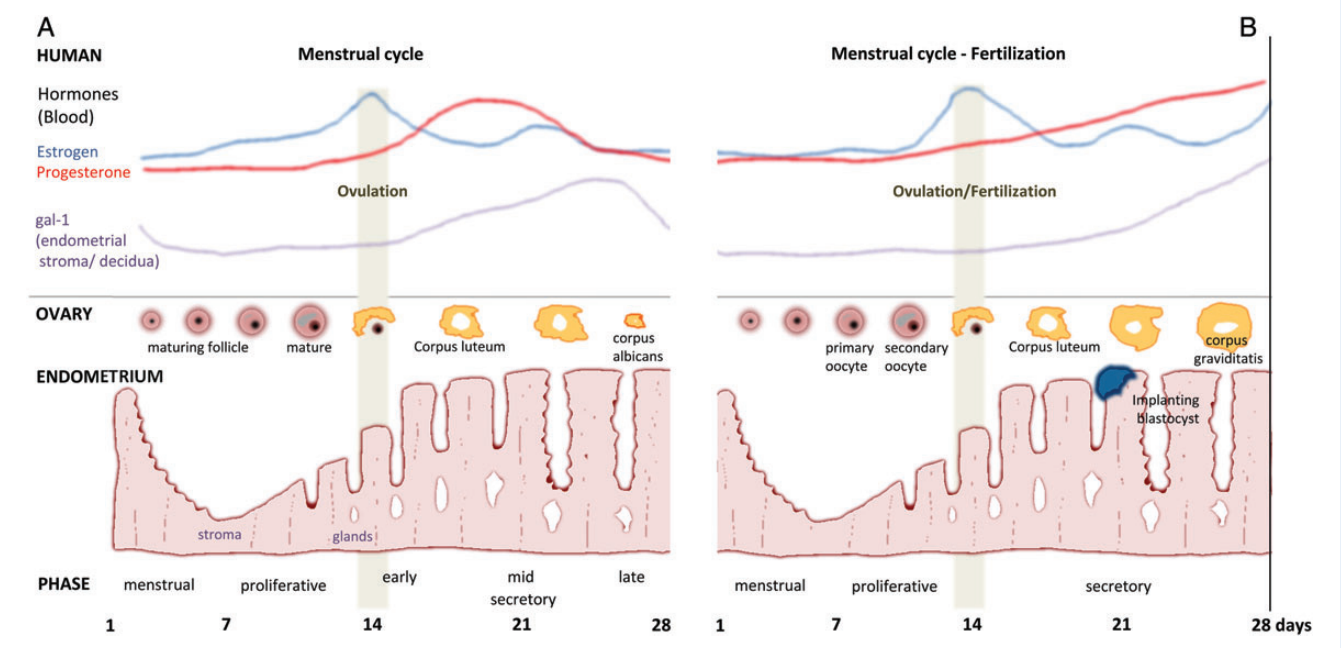

Figure 2 Dynamic changes in endometrial gal-1 expression characterize the menstrual cycle and pregnancy. In humans, gal-1 expression is up-regulated during the secretory phase of the menstrual cycle, concomitant with regulation by rising progesterone levels (**A**). Gal-1 expression in this period appears to be important for the transformation of endometrial stromal cells (decidualization) and establishment of the window of uterine receptivity. If implantation occurs (**B**), decidual gal-1 levels remain high and will be important in mediating maternal adaptations to pregnancy such as immunemodulation and vascular remodelling.

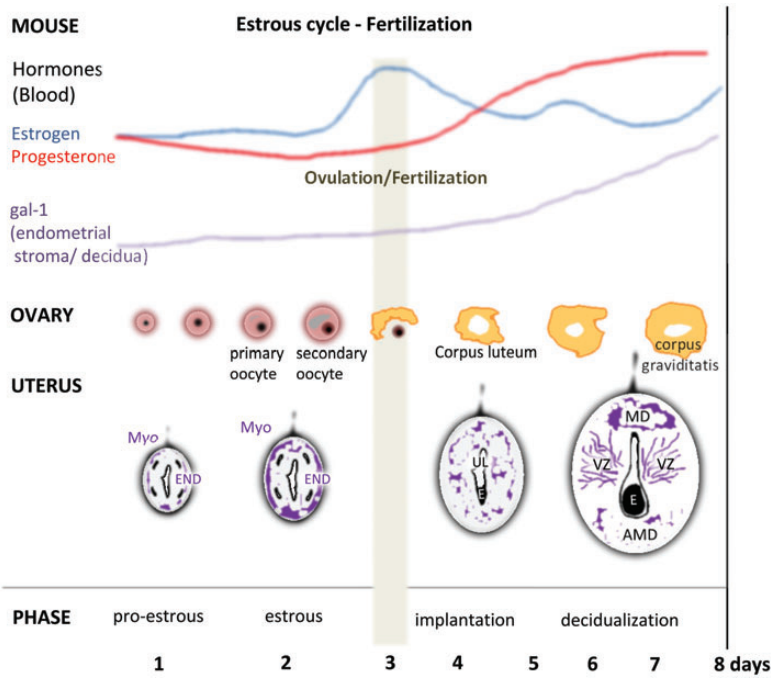

Figure 3 Steroid hormone regulation of endometrial gal-1 expression in mouse pregnancy. In the mouse uterus, the lectin is expressed in all tissues except the glandular and luminal epithelium, with particularly strong staining in decidualized stromal cells. Maternal gal-1 would play a similar role than in humans, mediating the structural changes related to decidualization, which in this case is triggered upon attachment of the embryo to the uterine lining. Myo, myometrium; END, endometrium; UL, uterine lumen; MD, mesometrial decidua; AMD, anti-mesometrial decidua; E, embryo; VZ, vascular zone.

luteolysis. Thus, the restored progesterone serum levels observed in stress-challenged pregnancies following supplementation with gal-1 may represent an as yet unknown compensatory mechanism to maintain PIBF synthesis and the immunological balance compatible with normal pregnancy.

The possible involvement of steroids in the regulation of expression of gal-1 in the placenta is not fully understood. On the one hand, high progesterone concentrations (i.e. 100 nM) have been found to stimulate the secretion of gal-1 by HTR-8/SVneo trophoblast cells, an extravillous trophoblast (EVT) cell line derived from human first trimester placenta immortalized with the SV40 large T antigen (Graham *et al.*, 1993). This effect was abrogated when HTR-8/SVneo cells were co-stimulated with progesterone in combination with RU486, suggesting a specific stimulation through the progesterone receptor in the EVT. On the other hand, we showed that stimulation with recombinant gal-1 (rgal-1) inhibits progesterone secretion by BeWo cells (Jeschke *et al.*, 2004), a choriocarcinoma cell line extensively used as an *in vitro* model mimicking the transformation of villous cytotrophoblasts (CTB) into syncytium. The reduction of progesterone by gal-1 was dose dependent and seemed to require functional binding to the Thomsen–Friedenreich (TF, Galbeta1-4GlcNAc and Galbeta1-3GalNAc) antigen. As will be discussed later, these results are consistent with the differential expression of gal-1 in the CTB and EVT lineages and argue for a role of this lectin as a paracrine signal modulating placental function. However, it remains to be determined how progesterone influences placentation by regulating gal-1 secretion/expression in the different trophoblast subsets in the human and mouse placenta. It will be interesting to understand how a key pregnancy hormone directs placental differentiation by regulating gal-1 locally.

Galectin-1 expression during early embryonic development

Expression of gal-1 has been detected at very early stages of human embryo development. For instance, Day 3 and Day 5 human embryos express gal-1 in the trophectoderm that subsequently gives rise to the differentiated trophoblasts of the placenta and in the inner cell mass (ICM) that eventually forms the definitive structures of the fetus (Tirado-Gonzalez *et al.*, 2013). In addition, we showed that human embryos secrete gal-1 into the medium in which they are cultured, suggesting that extracellular functions of this lectin could be important for uterine blastocyst attachment during the window of implantation. In support of this, we have shown that gal-1 binds mucin-1 (MUC1) via the TF epitope on glandular epithelial cells and endometrial epithelial apical surface tissue (Jeschke *et al.*, 2009). Interestingly, MUC1 and TF expression are up-regulated from the proliferative to the late secretory phase of the menstrual cycle, suggesting that embryonic-derived gal-1 may bind to endometrial MUC1 via the TF epitope during implantation (Jeschke *et al.*, 2009).

Though it is absent from the ICM, the mouse blastocyst also displays gal-1 expression in the trophectoderm and an involvement of this lectin in the attachment and invasion of the uterine wall during the process of implantation in this species has been assumed as well (Poirier *et al.*, 1992). Importantly, humans appear to be an exception in terms of MUC1 expression during the window of uterine receptivity, as in most species (including mice) this proteoglycan is anti-adhesive and is therefore down-regulated at the uterine luminal epithelium concomitant with implantation (Carson *et al.*, 1998). In this case, additional systems of attachment and implantation like the Syalil-Lewis a/x-selectin interaction might, at least in part, explain the finding that implantation can still occur in gal-1$^{-/-}$ and even double gal-1$^{-/-}$ gal-3$^{-/-}$ mutant mice (Colnot *et al.*, 1998).

Maternal galectin-1 and the adaptation to pregnancy

Since the pioneer research carried out by Bevan and Phillips in the 1990s (Bevan *et al.*, 1994; Phillips *et al.*, 1996), studies profiling gal-1 expression in the female reproductive tract have multiplied providing important insights into its function and emphasizing the idea that the balanced action of maternal and fetal sources of expression is most critical for healthy gestations. Maternal gal-1 has been implicated in the process of decidualization, i.e. the transformation of the endometrial stroma into a specialized tissue that supports trophoblast invasion and provides nourishment to the embryo before the establishment of the definite placenta. Although there are subtle differences between mammalian species, the hallmarks to this process are the differentiation of the stromal fibroblasts into large, rounded decidual cells accompanied by an extensive remodelling of the extracellular matrix driven by the concerted action of the steroid hormones estrogen and progesterone (Ramathal *et al.*, 2010). In humans, gal-1 expression is elevated during the secretory phase of the menstrual cycle when the endometrial stromal cells start to decidualize and further increased in the decidua of the first trimester of pregnancy (Bevan *et al.*, 1994; von Wolff *et al.*, 2005; Tirado-Gonzalez *et al.*, 2013). At term, the stromal cells still express gal-1 implicating a role in maintaining decidual cellular integrity (Bevan *et al.*, 1994). Mice differ from humans in that decidualization is triggered by the attachment of the embryo during implantation, but otherwise show a similar distribution of endometrial gal-1 expression. The lectin is expressed in all uterine tissues except the glandular and luminal epithelium, with particularly strong cytoplasmic staining in decidualized stromal cells (Phillips *et al.*, 1996). The mechanisms by which gal-1 may influence decidual development are still largely elusive, but are most likely to rely on its well-described role as a growth-regulatory protein (Camby *et al.*, 2006). Alternatively, the interaction of gal-1 with integrins, fibronectin and laminin suggests a role in extracellular matrix assembly and cell–matrix interactions in stromal cell transformation during decidualization (Ozeki *et al.*, 1995). In this regard, an aspect worth investigating is the potential involvement of gal-1-osteopontin interactions in the molecular control of decidualization (Moiseeva *et al.*, 2000). Both in mice and humans, osteopontin expression is dramatically increased with the progression of decidualization and shows a positive correlation with the recruitment of uterine NK (uNK) cells (von Wolff *et al.*, 2004; Herington and Bany, 2007; Qu *et al.*, 2008), which have for long been implicated in several processes critical for successful pregnancies such as the maintenance of decidual integrity and maternal immune tolerance. Given the selective expression of gal-1 observed in human uNK cell subsets (Koopman *et al.*, 2003), it is conceivable that an interplay with osteopontin may be involved in the regulation of their trafficking and function at the decidua during early stages of pregnancy.

Arguably the best studied function of gal-1 in the context of reproduction is its ability to support the maternal immune adaptation to pregnancy. The effects of gal-1 on components of the innate and adaptive immune system are consistent with a role as a tolerogenic and anti-inflammatory signal, being strongly implicated in mechanisms of tumour immune evasion and autoimmune diseases (Camby *et al.*, 2006). Likewise, the decidua of pregnancy constitutes an interesting physiological system in which inflammation and immune cell activation are delicately modulated by gal-1 to prevent damage to the developing fetus while retaining the ability to protect the mother from infections. Thus, gal-1 appears to be essential for maintaining the balance between pro-inflammatory T helper (Th)-1 cytokines and tolerogenic Th2 cytokines like IL-4, IL-5 and IL-10 needed for a successful pregnancy (Lin *et al.*, 1993; Piccinni *et al.*, 1998; Blois *et al.*, 2004; Blois *et al.*, 2007). This effect was first demonstrated in a mouse model of immunological abortions, in which exposure to stress boosts the fetal loss rate by causing a predominance of abortogenic Th1 cytokines (Blois *et al.*, 2004; Blois *et al.*, 2007). Stress-challenged mice exhibited a remarkable decrease of decidual gal-1 expression, and supplementation with gal-1 recombinant during early pregnancy was able to prevent fetal rejection by restoring the Th1 to Th2 cytokine ratio of decidual mononuclear cells. Mechanistically, gal-1 appears to restore the immunological balance in the decidua by virtue of its ability to induce the differentiation of tolerogenic $CD11c^+$ dendritic cells (DCs), which in turn promote the expansion of IL-10 producing $CD4^+CD25^+$ regulatory T cells (Blois *et al.*, 2007). In line with these findings, *LGALS1* null mice display exacerbated Th1 and Th17 responses and a higher frequency of immunogenic DC (Toscano *et al.*, 2007; Ilarregui *et al.*, 2009), and although they reproduce normally upon syngeneic matings, these mice show increased fetal loss rates and an enhanced susceptibility to stress-induced abortions in allogeneic pregnancies compared with their wild-type counterpart (Blois *et al.*, 2007; Friebe *et al.*, 2011). Importantly, the tolerogenic signals involved in pregnancy maintenance may additionally derive from gal-1 expressing immune cells themselves, as has been demonstrated for human uNK cell subsets (Koopman *et al.*, 2003). Thus, uNK cell derived gal-1 has been shown to promote apoptosis of decidual activated T cells, which present a glycophenotype compatible with sensitivity to this lectin (Kopcow *et al.*, 2008). Furthermore, unlike peripheral NK cells, uNK cells selectively express gal-1 and type 2 β-1,6-*N*-acetylglucosaminyl transferase (C2GNT), the glycosylation enzyme required to initiate formation of its specific ligands, implying a role for this lectin as an autocrine signal down-modulating the cytotoxic potential of this cell subset (Kopcow *et al.*, 2008). Recently, we demonstrated that uNK cells shape the immunological functions of the DC *in vivo* (Tirado-Gonzalez *et al.*, 2012), which is consistent with the involvement of a functional DC-NK cell cross talk in the maintenance of pregnancy. The cross talk between uNK cells and DC has been shown to enhance the induction of regulatory T cells by DC and the proliferation and differentiation of IL-10-producing NK cells (Lin *et al.*, 2008; Vacca *et al.*, 2010), processes that may well be triggered by interactions of gal-1 with specific carbohydrate ligands present in both cell subsets. Thus, these studies leave an open question as to the involvement of gal-1 in the modulation of cross-regulatory interactions between NK cells and DC in the early pregnancy decidua.

Placental galectin-1 and pathways of trophoblast cell differentiation

Mice and humans share the characteristics of a haemochorial placenta, in which the maternal blood comes into direct contact with the chorion, but their placentation differs in morphogenesis and endocrine functions [reviewed in (Malassine *et al.*, 2003)]. In humans, gal-1 expression is evidenced very early in the trophectoderm and subsequently shows a differential distribution in trophoblast cell lineages, arguing for its function as a key protein in the differentiation of trophoblasts during placentation. Briefly, this process is characterized by the differentiation of villous CTB stem cells along two possible pathways, both of which seem to be influenced by gal-1: either by fusing to give rise to the outermost syncytiotrophoblast layer of the placenta or along an invasive route, in which they form EVT columns that physically attach the placenta to the uterus (Aplin, 1991). In the first trimester placenta, gal-1 localizes to villous CTB cells where it appears to play a significant role in promoting their syncytium formation (Fischer *et al.*, 2010; Tirado-Gonzalez *et al.*, 2013). Indeed, *in vitro* studies performed in BeWo choriocarcinoma cells and human CTB have shown that gal-1 stimulates syncytin expression and down-regulates β-catenin and E-cadherin, which are indicators of cell fusion during syncytial differentiation (Fischer *et al.*, 2010). Interestingly, although the differentiation into syncytiotrophoblast appears to be accompanied by a loss of gal-1 expression (Bevan *et al.*, 1994; Tirado-Gonzalez *et al.*, 2013), these cells remain responsive to the lectin showing a down-regulation of progesterone and hCG secretion upon supplementation of exogenous gal-1 (Jeschke *et al.*, 2004). In contrast, the EVT arising from villous CTB that undergo the invasive pathway display an increased gal-1 expression (Aplin, 1991; Tirado-Gonzalez *et al.*, 2013). The differential gal-1 expression observed in the syncytium and EVT lineages may either reflect different functional requirements for this lectin or the existence of two distinct pools of villous CTB progenitors (James *et al.*, 2005). Of interest, those EVT that subsequently invade the decidua (i.e. interstitial EVT) display the highest gal-1 expression (Bevan *et al.*, 1994; Vicovac *et al.*, 1998), implying an important role for this lectin in the modulation of the invasive pathway of trophoblast differentiation. Indeed, the ability of gal-1 to stimulate the adhesion and invasion of trophoblast cells has been demonstrated *in vitro* using HTR-8/SVneo cells and primary EVT cultured in Matrigel, further showing that this function relies on the interplay between intra- and extracellular sources of gal-1 (Kolundzic *et al.*, 2011). Adding to the evidence on the importance of gal-1 as an endogenous signal-modulating EVT differentiation, we have recently demonstrated that treatment of the HIPEC65 EVT cell line with an oligonucleotide complementary to *LGALS1* significantly down-regulated the expression of the human leukocyte antigen (HLA)-G1 and -G2 isoforms and their soluble counterparts HLA-G5 and G6 (Tirado-Gonzalez *et al.*, 2013). HLA-G expression is considered to be a molecular signature of invasive trophoblasts that represents a chief mechanism promoting feto-maternal tolerance by virtue of its suppressive effects on $CD8^+$ T lymphocytes, NK cells and other immune cell subsets (McMaster *et al.*, 1995; Hunt *et al.*, 2005). Taken together, these results provide an interesting example of how a single molecule can orchestrate the differentiation programme of trophoblast cells and encourage further research on the role of gal-1 in diseases like pre-eclampsia which has been linked to decreased expression of HLA-G concomitant with insufficient placentation.

In contrast to humans, the definitive structure of the mouse chorioallantoic placenta is established during mid-gestation and consists of a labyrinthine and junctional zone separated from the maternal decidua by a trophoblast giant cell (TGC) layer (Malassine *et al*., 2003). At the beginning of placentation, the trophoblast differentiates into proliferating trophoblasts of the extra-embryonic ectoderm and ectoplacental cone where the expression of gal-1 points to its involvement in the differentiation process as assumed in humans (Phillips *et al*., 1996). However, the studies investigating the participation of gal-1 in developmental processes associated with mouse placentation are mainly descriptive, only allowing a speculative inference of its role based on the expression patterns in the different trophoblast lineages. For instance, during early gestation, mouse TGCs mediate blastocyst attachment and invasion into the uterine lining and may thus be considered a functional analogue to the human EVTs forming the anchoring villi (Hu and Cross, 2010). However, TGCs are characterized by low cytoplasmic gal-1 expression (Hirabayashi and Kasai, 1984; Phillips *et al*., 1996), which is more consistent with their function during later stages supporting the endocrine activity of mouse placental tissue (i.e. secretion of steroid hormones and prolactin-related cytokines). In contrast, trophoblasts in the labyrinth and junctional zone strongly express gal-1 in their nucleus and cytoplasm, whereas the vascular spaces in the labyrinth are also positively stained for gal-1 (Hirabayashi and Kasai, 1984; Phillips *et al*., 1996). Since in the labyrinth zone, oxygen and nutrients are exchanged between maternal and fetal blood vessels, gal-1 may support the cell–cell interactions needed for exchange processes. The expression of gal-1 has also been reported in glycogen cells and spongiotrophoblasts forming the junctional zone (Hirabayashi and Kasai, 1984; Phillips *et al*., 1996), but its functional significance here remains largely elusive. As the mouse constitutes an important model system for the study of mechanisms underlying human pregnancy disorders such as spontaneous abortion and pre-eclampsia, a re-examination of the role of gal-1 during mouse placentation may provide important insights on the molecular pathways driving the differentiation and functional properties of trophoblast cells.

Galectin-1 and angiogenesis

Importance of angiogenesis during gestation

Pregnancy success is critically dependent on several vascular processes coordinated in a spatio-temporal manner at the maternal–fetal interface. At early stages, hormonally mediated adaptations of the endometrial vasculature enable the implantation of the embryo into a richly vascularized receptive uterus. This is accompanied by expansion and *de novo* formation of blood vessels during decidualization to ensure the embryo will be supplied with oxygen and nutrients before the establishment of the definite placenta. As pregnancy progresses, the decidual and placental vasculature continue to be remodelled to enable blood flow to the increasing metabolic demands of the fetus, and disturbances in these processes are often associated with adverse pregnancy outcomes, i.e. pre-eclampsia, intrauterine growth restriction or pre-term delivery (Reynolds *et al*., 2006).

Angiogenesis, i.e. the formation of new blood vessels from pre-existing capillaries, is a process of pivotal importance for normal female reproduction as it influences several functions such as follicular development and luteinization, uterine receptivity and placental development. During mouse pregnancy, for instance, the administration of a single dose of anti-angiogenic compounds (e.g. AGM-1470) precludes implantation and placentation, resulting in early pregnancy loss (Klauber *et al*., 1997). Pregnancy-associated vascular responses are mainly driven by trophoblast-derived signals in a manner similar to how tumours recruit a blood supply to support their own growth. In mice, placental TGCs mediate contact with maternal tissues and show the ability to produce a remarkable variety of hormones, angiogenic and vasoactive factors that contribute to placental angiogenesis and vasculogenesis (Cross *et al*., 2002). Likewise, human CTB and especially invasive EVT are also considered key regulators of angiogenesis, as they have been shown to promote the expression of vasoactive proteins in adjacent decidual cells (Hess *et al*., 2007). However, at least in mice, early angiogenic responses prior to the establishment of interactions between the trophoblast and the vasculature appear to be mediated by immune cell subsets infiltrating the decidual tissue (Croy *et al*., 2012). Thus, we and others have recently demonstrated that DC and NK cells are important modulators of angiogenesis necessary for proper formation of the decidual vascular bed during early pregnancy (Plaks *et al*., 2008; Degaki *et al*., 2012; Barrientos *et al*., 2013).

In the multiplicity of all-known angiogenic pathways, the interplay between the classical VEGF receptor 2 (VEGFR2) and its co-receptor neuropilin (NRP)-1 appears to be the most important with regard to the involvement of gal-1 in the angiogenesis process. NRP-1 is an endothelial cell receptor, specifically binding the human $VEGF_{165}$ and mouse $VEGF_{164}$ isoforms, which upon ligation has been shown to enhance the activation and signalling of the VEGFR2 (Soker *et al*., 1998; Halder *et al*., 2000). Interestingly, *in vitro* studies have shown that gal-1 selectively binds NRP-1 via its CRD to promote adhesion and migration of endothelial cells, and that stimulation with exogenous gal-1 significantly increases VEGFR2 phosphorylation (Hsieh *et al*., 2008). Furthermore, the enhanced migratory activity induced by gal-1 was abrogated upon si-RNA silencing of VEGFR2 expression, supporting the notion that the pro-angiogenic activity of this lectin results from its functional cooperation with the VEGF pathway.

VEGF is differentially expressed in tissue compartments of the mouse maternal–fetal interface that display an increased angiogenic activity (Halder *et al*., 2000). The pivotal importance of VEGF-mediated angiogenesis during early mouse pregnancy was recently demonstrated by *in vivo* studies showing that in contrast to the inhibition of VEGFR1 or VEGFR3, a single dose of the VEGFR2-neutralizing antibody DC101 during the implantation period resulted in complete fetal loss due to a severely reduced decidual vascularization (Douglas *et al*., 2009). The levels of VEGFR2 and NRP-1 increase during the first days of gestation and show a largely overlapping localization, being highly expressed in stromal endothelial cells during implantation and decidualization (Halder *et al*., 2000) (Fig. 4). Analysis of the expression of VEGF, VEGFR2 and NRP-1 throughout human pregnancy showed that all three factors are preferentially expressed during the first trimester and localize to the vessels, stroma and glands of the decidua as well as CTB in the chorionic villi (Fig. 4), implying a similar role in the promotion of early vascular responses (Sugino *et al*., 2002; Baston-Buest *et al*., 2011). Interestingly, while at least in mice cross-linking experiments have shown binding of $VEGF_{165}$ to both NRP-1 and VEGFR2 present in decidual endothelial cells (Halder *et al*., 2000), a direct involvement of gal-1 in the modulation of pregnancy-associated angiogenesis, either alone or through its cooperative action with the VEGF pathway, could be hypothesized.

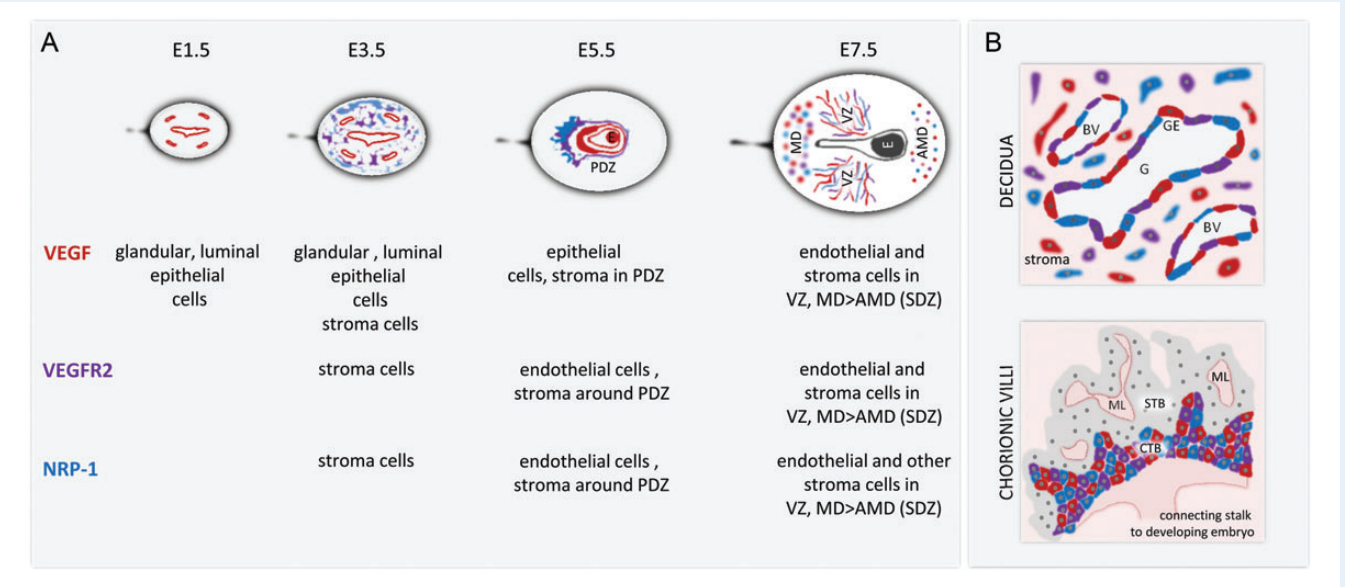

Figure 4 Distribution of VEGF, VEGFR2 and NRP-1 during early pregnancy. (**A**) In mouse pregnancy, VEGF expression is detectable as early as embryonic day (**E**) 1.5, being localized to tissue compartments with high angiogenic activity. Its receptors VEGFR2 and NRP-1 increase during the first days of gestation and show a largely overlapping localization, being highly expressed in stromal endothelial cells during implantation and decidualization. (**B**) In humans, overlapping expression of these factors implies a similar role in the promotion of early vascular responses. VEGFR2 and NRP-1 are mainly found on decidual blood vessels during the first trimester, whereas VEGF production appears to depend on both maternal (decidual stroma and glands) and embryonic sources (villous CTB). PDZ, primary decidua zone; SDZ, secondary decidua zone; MD, mesometrial decidua; AMD, antimesometrial decidua; E, embryo; VZ, vascular zone; BV, blood vessel; G, gland; GE, gland epithelium; STB, syncytiotrophoblast; CTB, cytotrophoblast; ML, maternal lacuna.

Pro-angiogenic properties of galectin-1

Aside from pregnancy, the role of gal-1 in the activation and execution of angiogenesis has been well documented in a variety of physiopathological settings (Thijssen *et al.*, 2007). Several studies have shown that hypoxia, possibly via multiple cell type-dependent pathways, can induce the expression and secretion of gal-1 (Le *et al.*, 2005; Zhao *et al.*, 2010; Zhao *et al.*, 2011). Furthermore, endothelial cells bind and sequester gal-1 from their environment, which results in triggering of H-Ras signalling indicative of cell activation (Thijssen *et al.*, 2010). As described above, Hsieh *et al.* (2008) found that gal-1 facilitates VEGFR2 signalling in endothelial cells, which was attributed to carbohydrate-mediated binding of gal-1 to NRP-1. Thus, cells that secrete gal-1 can induce the angiogenic activity of endothelial cells, which is accompanied by enhanced proliferation, migration and tube formation *in vitro* as well as by increased angiogenesis *in vivo* (Hsieh *et al.*, 2008; Thijssen *et al.*, 2010; Ito *et al.*, 2011).

Besides acting as a pro-angiogenic growth factor, gal-1 is also described as an early marker of endothelial cell activation (Thijssen *et al.*, 2006; Thijssen *et al.*, 2008). This is exemplified by the observation that other than hypoxia, different stimuli including lipoproteins, lipopolysaccharides and cytokines induce endothelial expression of gal-1 (Baum *et al.*, 1995; Perillo *et al.*, 1995; He and Baum, 2006). In addition, endothelial gal-1 is frequently increased in pathologies that are characterized by enhanced angiogenesis, including cancer (Schulkens *et al.*, 2012). While these observations suggest an important role of gal-1 during angiogenesis, gal-1-deficient mice were found to lack an altered vascular phenotype and to be viable and fertile (Poirier and Robertson, 1993). Furthermore, Le Mercier *et al.* (2008) did not observe any effect on capillary network formation following gal-1 knockdown in endothelial cells suggesting only a minor function for the lectin in angiogenesis. On the other hand, we and others found that knockdown of endothelial gal-1 expression inhibits endothelial cell proliferation and migration (Thijssen *et al.*, 2006; Hsieh *et al.*, 2008; Thijssen *et al.*, 2010). Furthermore, silencing of gal-1 was shown to induce vascular defects during developmental angiogenesis in zebrafish embryos pointing towards hampered vascular guidance (Thijssen *et al.*, 2006). In addition, gal-1-deficient mice show a clear defect in pathological angiogenesis (Thijssen *et al.*, 2006; Thijssen *et al.*, 2010; Banh *et al.*, 2011) and several studies have shown that interfering with gal-1 using different approaches, including RNA interference, blocking peptides or antibodies and competing carbohydrates, affects endothelial cell function and angiogenesis (Sorme *et al.*, 2003; Thijssen *et al.*, 2006).

Recently, we showed that pro-angiogenic properties of gal-1 are relevant for vascular development at the fetal–maternal interface. Indeed, the administration of gal-1 promotes embryo survival in a model of reduced vascular expansion via VEGFR2 signalling (Freitag *et al.*, 2013). On the one hand, gal-1 can directly regulate angiogenesis at early stages of pregnancy by enhancing the expression of classical pro-angiogenic factors [e.g. angiogenin, chemokine (C-X-C motif) ligand 16 (CXCL16), heparin-binding epidermal growth factor (HB-EGF)] and also by promoting blood vessel activation within the vascular zone of the decidua. On the other hand, this lectin enhances VEGF bioavailability and expression of proteins involved in matrix remodelling [e.g. matrix metallopeptidase 9 (MMP-9), tissue inhibitor of metalloproteinase 1 (TIMP-1)], which could in turn indirectly promote angiogenesis.

These findings designate a novel function for gal-1 as a regulator of angiogenesis and vascular development during early stages of gestation.

Galectin-1 signalling at the maternal–fetal interface

gal-1 exerts multiple biological functions including cell differentiation, proliferation, growth, apoptosis, migration, adhesion, transformation, cell–cell and cell–matrix interactions and even pre-mRNA splicing (Camby *et al.*, 2006, Nakahara and Raz, 2006). These functions influenced by gal-1 play important roles in the orchestration of developmental processes at the feto-maternal interface, such as the transformation of endometrial cells during decidualization, the apoptosis of activated T cells to mediate maternal tolerance and the differentiation of trophoblasts into distinct phenotypes with different invasive and functional properties (von Wolff *et al.*, 2005; Kopcow *et al.*, 2008; Kolundzic *et al.*, 2011; Tirado-Gonzalez *et al.*, 2013). However, the study and identification of receptors for gal-1 is only emerging and many possible interactions for the modulation of cellular functions remain to be elucidated. Here, we provide a brief summary of identified gal-1-binding partners and the intracellular signalling cascades activated by them in the context of pregnancy.

Cell-surface receptors and ECM glycoproteins binding galectin-1

While gal-1 was originally defined as β-galactoside-binding lectin, it has become increasingly clear that it can also engage in protein–protein interactions. With few exceptions, the lectin activity of gal-1 is observed in the extracellular milieu, while the protein–protein interactions concern its intracellular functions (Camby *et al.*, 2006). Most of the extracellular-binding partners for gal-1 identified to date are glycoconjugates rich in the ubiquitous disaccharide *N*-acetyllactosamine (Gal-β1-3/4 GlcNAc, known also as LacNAcII). The arrangement of lactosamine disaccharides in branched repeating chains is responsible for high-avidity lectin activity, but binding of gal-1 to single lactosamine units is also possible though with relatively low levels of affinity (Ahmad *et al.*, 2004). Importantly, the binding avidity of dimeric gal-1 is enhanced when its branched ligands are surface bound as on cell membranes or in the ECM (He and Baum, 2004; Leppanen *et al.*, 2005).

Binding of gal-1 to ECM components may play an important role for the establishment of cell–cell and cell–matrix interactions during implantation, decidualization and placentation (Table I). Thus, a dose- and β-galactoside dependent binding of the lectin to ECM proteins has been demonstrated in vascular smooth muscle cells with the following order of affinity: laminin > cellular fibronectin > thrombospondin > vitronectin > osteopontin (Moiseeva *et al.*, 2000; Moiseeva *et al.*, 2003a, b). Laminin and fibronectin are highly glycosilated proteins containing bi- and tetraantennary poly-*N*-lactosamines that demonstrate dynamic expression in relation to the morphological differentiation of endometrial stroma during human and rodent early pregnancy (Kayisli *et al.*, 2005; Kaloglu and Onarlioglu, 2010). Furthermore, anti-laminin antibodies as identified in mice immunized prior to pregnancy as well as in patients suffering from endometriosis and autoimmune disorders have been linked to infertility and spontaneous abortion (Qureshi *et al.*, 2000; Inagaki *et al.*, 2005). While these findings imply an important role for these proteins during the establishment of pregnancy, the functional relevance of their potential interactions with gal-1 remain largely elusive. Of the mentioned ECM components, binding of gal-1 to osteopontin offers interesting perspectives given the variety of functions ascribed to this protein in the context of reproduction. Osteopontin (also known as secreted phosphoprotein 1, SPP1) is the most highly up-regulated ECM molecule/cytokine during human uterine receptivity, and *Spp1* null mice manifest increased pregnancy loss rates when compared with wild-type counterparts during mid-gestation (Carson *et al.*, 2002; Weintraub *et al.*, 2004). Maternal osteopontin expression appears to play an important role during implantation and decidualization, as the protein is detected mainly in the decidual stroma and the luminal epithelium of most species where it has been found to mediate contact with the trophectoderm of the implanting blastocyst (White *et al.*, 2006; Kim *et al.*, 2010). Furthermore, *in vitro* studies have shown that phosphorylated SPP1 can significantly enhance the migration of human trophoblast cells (Al-Shami *et al.*, 2005), suggesting a role in trophoblast invasion that is further supported by the observation that the expression of this protein in the EVT and syncytium is significantly reduced during the course of pre-eclampsia (Gabinskaya *et al.*, 1998; Xia *et al.*, 2009). These findings, together with the cytokine-like properties of SPP1 and its demonstrated role as a potent chemotactic for immune cells (Weber and Cantor, 1996; Johnson *et al.*, 2003), encourage further investigations on its physical interaction with gal-1 in the context of pregnancy.

Among cell-surface binding partners described for gal-1, integrins appear to play important roles during pregnancy due to their influence in a variety of biological processes including apoptosis, invasion, proliferation and gene expression. Interestingly, many of the ECM components described as receptors for gal-1 (i.e. laminin, fibronectin, osteopontin) are also reported to bind integrins at the fetal maternal–interface (Johnson *et al.*, 2001; Kayisli *et al.*, 2005; Erikson *et al.*, 2009), creating a complex network of cell–cell and cell–matrix interactions involving these proteins that may preclude the identification of functions mediated by specific binding of gal-1 to integrins. Nevertheless, gal-1 binding to the b1 subunit of integrin was demonstrated in EVT, causing the phosphorylation of focal adhesion kinase to modulate cell–matrix interactions during trophoblast invasion (Moiseeva *et al.*, 2003a, b; Shyu *et al.*, 2011). Besides integrins, the best characterized cell-surface partner for gal-1 in the context of pregnancy is probably the TF epitope-carrying glycoprotein, MUC1. In human endometrium, MUC1 is abundant at the luminal epithelial surface in the receptive phase and experiences a loss of keratan sulphate chains concomitant with implantation. MUC1 carries the TF epitope on the apical surface of epithelial cells, and post-translational modifications of the protein result in incomplete glycosilation and enhanced exposure of the antigen as observed in tumour cells (Springer *et al.*, 1990). Binding of gal-1 to the TF epitope appears to be implicated in two main processes during human pregnancy: (i) attachment of the trophectoderm to the endometrium within the window of implantation (Jeschke *et al.*, 2009) and (ii) the differentiation of villous CTB into syncytium at the placenta (Fischer *et al.*, 2010). The latter function has even been characterized in terms of the signalling pathways activated upon gal-1 ligation, as will be discussed in the following section. Interestingly, placental overexpression of MUC1 in severe pre-eclampsia has been associated with decreased invasion of the EVT by interfering with cell–matrix adhesion and the pro-migratory activity mediated by the b1 integrin subunit (Shyu *et al.*, 2011). Thus, further investigation of the interaction between gal-1 and its cell-surface partners, MUC1

Table I Galectin-1 receptors identified at the maternal–fetal interface.

Receptor	Function	Distribution at the fetal maternal interface	Influence on pregnancy
Laminin Fibronectin	ECM component	Basal membrane of blood vessels, decidua, placental bed	Decidualization, angiogenesis
Thrombospondin	ECM component	Endometrial stroma, myometrium	Angiogenesis, parturition
Osteopontin/ SPP1	ECM component, cytokine	Uterine luminal epithelium, trophoblast (EVT and STB); macrophages and uNK cells	Blastocyst attachment, trophoblast migration, leukocyte trafficking
Integrin β1	Adhesion molecule, signalling	EVT columns Glandular epithelium, decidual stroma	Trophoblast invasion* Tissue remodelling during receptivity, trophoblast invasion
Neuropilin-1	Cell-surface receptor	Endothelial cells	Angiogenesis
MUC1/TF epitope	Cell-surface glycoprotein	Uterine luminal epithelium Villous CTB	Receptivity, blastocyst attachment* Trophoblast differentiation*, hormonal secretion*

Brief summary of components of the rodent and human maternal–fetal interfaces identified as gal-1 receptors, and their putative functions in the context of pregnancy. Processes denoted with asterisks (*) are confirmed to be mediated by gal-1 ligation. EVT, extravillous trophoblast; STB, syncytiotrophoblast; CTB, cytotrophoblast.

and integrin, in different trophoblast lineages may provide important insights into the pathogenesis of life-threatening pregnancy disorders such as pre-eclampsia.

Intracellular signalling pathways activated by galectin-1

Effect of gal-1 in syncytium formation

Syncytial fusion is an essential process for the maintenance of syncytiotrophoblast, a terminally differentiated trophoblast layer, responsible for the majority of biological functions assigned to the placenta. This cellular process is dependent on a precise series of membrane-mediated events (Douglas and King, 1990) and is regulated by multiple factors including cytokines, fusion proteins, proteases and cytoskeletal proteins (Yang *et al.*, 2003). Cadherins, members of a superfamily of integral membrane glycoproteins, are likely molecular players that mediate end differentiation and fusion of the CTB (Coutifaris *et al.*, 1991). The ability of cadherins to mediate cell–cell interactions is dependent on interaction with at least three cytoskeletal-associated proteins known as α-, β- and γ-catenin. During the aggregation, differentiation and fusion of human CTB cells, there is a significant down-regulation of E-cadherin and β-catenin expression (Getsios *et al.*, 2000). In this context, we have shown that gal-1 is able to enhance fusion of BeWo cells, a trophoblast-derived choriocarcinoma cell line used to mimic syncytium formation *in vitro* by down-regulating the expression of β-catenin and E-Cadherin (Fischer *et al.*, 2010) (Fig. 5). This is in agreement with the role of gal-1 in initiating myoblast fusion and the reduced myofibre formation observed in gal-1-null mice (Watt *et al.*, 2004; Georgiadis *et al.*, 2007).

Regulation of trophoblast cell growth by gal-1

Interestingly, the extracellular effect of gal-1 on syncytium formation on the BeWo cell line is accompanied by inhibition of proliferation evidenced by the decreased Ki67 expression and BrdU incorporation (Jeschke *et al.*, 2006; Fischer *et al.*, 2010). The anti-proliferative effect of gal-1 results from the inhibition of the Ras-MEK-ERK pathway (Fischer *et al.*, 2005) and differentiation of CTB into syncytiotrophoblast is characterized by a reduced ERK1/2 and p38 MAPK activities (Kita *et al.*, 2003; Daoud *et al.*, 2005). Recently, we have demonstrated that phosphorylation of mitogen-activated protein kinase (MAPK)/ERK2 is reduced upon gal-1 treatment of BeWo cells (Fischer *et al.*, 2011). Furthermore, up-stream signals of ERK1/2 and p38 MAPK, such as RE aranged during transfection (RET) and Janus Kinase (JAK)2, were down-regulated by gal-1 treatment (Fischer *et al.*, 2009), suggesting that this lectin binds to RET through glycosylphosphatidylinositol-linked cell-surface proteins via TF which ultimately results in growth inhibition (Fig. 5). However, gal-1 treatment induces the phosphorylation of VEGFR3, which has been implicated in cellular survival under stress conditions (Shibuya and Claesson-Welsh, 2006). Thus, gal-1 can influence cell survival via VEGFR3 and regulate cell growth via the Ras-MEK-ERK pathway (Fig. 5), contributing to the syncytium formation on the anchoring villi.

In contrast, intracellular gal-1 does not seem to be involved in cell growth regulation as we have shown that the administration of a specific oligodeoxynucleotide against gal-1 did not alter the growth of the human invasive proliferative extravillous CTB (HIPEC) 65 cell line (Tirado-Gonzalez *et al.*, 2013). This evidence allows us to speculate that extracellular and intracellular gal-1 activities on different trophoblast cell subsets do not both involve the promotion of cell growth. On the contrary, this lectin seems to highly regulate cell transformation and differentiation during the placentation process.

Role of gal-1 in HLA-G expression

HLA-G, a class Ib molecule, plays a role in maternal tolerance of trophoblast cells (Le Bouteiller *et al.*, 2007). In this context, HLA-G expression on EVTs protects them from lysis by NK cells (Rouas-Freiss *et al.*, 1997), which is of critical importance, since during normal pregnancy trophoblasts closely associate with NK cells at the implantation site (Loke and King, 1997). Among the factors that play a role in placentation and invasion of trophoblasts, gal-1, both inside and outside EVT cells, modulates HLA-G expression. This results from the activation of the non-receptor tyrosine kinases (PTKs)-Ras-mitogen-activated protein kinase (Ras-MAPK) pathway and consecutive transcription induction of the Sp1-binding site of the HLA-G promoter is important to gal-1 responsiveness (Tirado-Gonzalez *et al.*, 2013). In addition, a progesterone receptor response element (PRE) is also located along the HLA-G gene

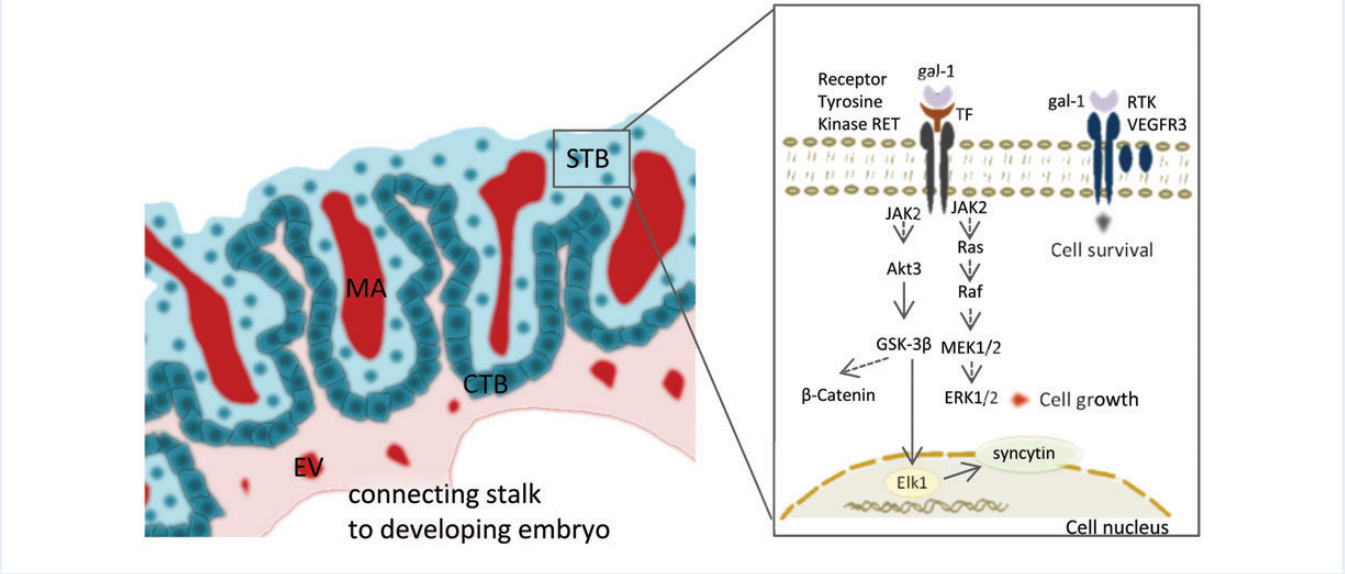

Figure 5 Signalling pathways activated by gal-1 in trophoblast cell growth and syncytium formation. Gal-1 binds the TF epitope in villous CTB leading to a significant down-regulation of E-cadherin and β-catenin expression and induction of syncytin features of syncytial fusion. This is accompanied by decreased proliferation due to inhibitory effects of the lectin on the Ras-MEK-ERK pathway and upstream signals delivered by JAK2 and RET. However, growth regulation results from a balance between these pathways and those mediated upon gal-1 ligation of the VEGFR3, which has been implicated in the promotion of cellular survival under stress conditions. CTB, cytotrophoblasts; STB, syncytiotrophoblast; MA, maternal arteriole; EV, embryonic vessels; RTK, receptor tyrosine kinase.

promoter (Yie *et al.*, 2006), and thus the cross talk between gal-1 and progesterone discussed in this review could be relevant for the regulation of HLA-G expression on EVT cells.

Galectin-1 in reproductive disorders

The adaptation of the maternal immune system to pregnancy is believed to play a key role during early stages of mammalian gestation. In this context, gal-1 is deeply involved in modulating innate and adaptive immune responses (Camby *et al.*, 2006) and also in regulating different process during pregnancy (Blois *et al.*, 2007; Tirado-Gonzalez *et al.*, 2013). Therefore, it is reasonable to assume that gal-1 expression during early gestation could be used to advance the prognosis and diagnosis of poor pregnancy outcomes. In this section, we discuss the association between gal-1 expression and pregnancy outcome in the context of human reproductive disorders.

Spontaneous abortion

Being a common complication of pregnancy, spontaneous abortion is characterized by the loss of a fetus prior to 20 weeks of gestation. Approximately 15–20% of clinically recognized pregnancies are spontaneously aborted, most of them before Week 13 (Boivin *et al.*, 2007; Ombelet *et al.*, 2008). Fetal chromosomal abnormalities are the major cause of spontaneous abortion contributing to ~50–60% of the cases (Nagaishi *et al.*, 2004). However, anatomic anomalies, endocrine or immune problems, coagulation protein defects, nutritional deprivation and environmental factors (such as psychological stress) represent other aetiologic factors of spontaneous abortion (Boivin *et al.*, 2007; Ombelet *et al.*, 2008). A proteomic study identified that gal-1 expression is deregulated in placental villous obtained from spontaneous abortion patients when compared with healthy normal gestation (Liu *et al.*, 2006). In addition, we have also shown that decidual gal-1 expression is diminished in patients with spontaneous abortion (Tirado-Gonzalez *et al.*, 2013). Besides showing that gal-1 is deregulated in diagnosed spontaneous abortion patients, the latter study identified gal-1 circulating levels as a novel biomarker for predicting pregnancy outcome, being more sensitive than serum β-hCG levels (Tirado-Gonzalez *et al.*, 2013). At present, sonographic parameters (e.g. fetal heart rate, crown-rump length) and maternal serum β-hCG levels are used for prognosis and diagnosis of spontaneous abortion (Reis *et al.*, 2002). However, the use of sonographic parameters for prenatal diagnosis and treatment is controversial (Reis *et al.*, 2002). Therefore, we propose that inclusion of serum gal-1 levels, either alone or in combination with the other parameters, could improve the diagnosis of poor pregnancy outcome.

Recently, another study showed that serum gal-1 levels are decreased in patients with recurrent spontaneous abortion (RSA) (Ramhorst *et al.*, 2012). This pregnancy disorder is different from spontaneous abortion, since it is characterized by three consecutive pregnancy losses prior to 20 weeks and affects ~1–2% of women (Rai and Regan, 2006). Decreased gal-1 peripheral levels were accompanied by an increased concentration of anti-gal-1 auto-antibodies in RSA patients (Ramhorst *et al.*, 2012). In this context, we have determined the frequency of anti-gal-1 auto-antibodies during normal pregnancy including patients in the first, second and third trimester. As shown in Fig. 6, anti-gal-1 auto-antibodies increase their levels as pregnancy progresses, reaching a peak at term. When we analysed the anti-gal-1 auto-antibodies between women

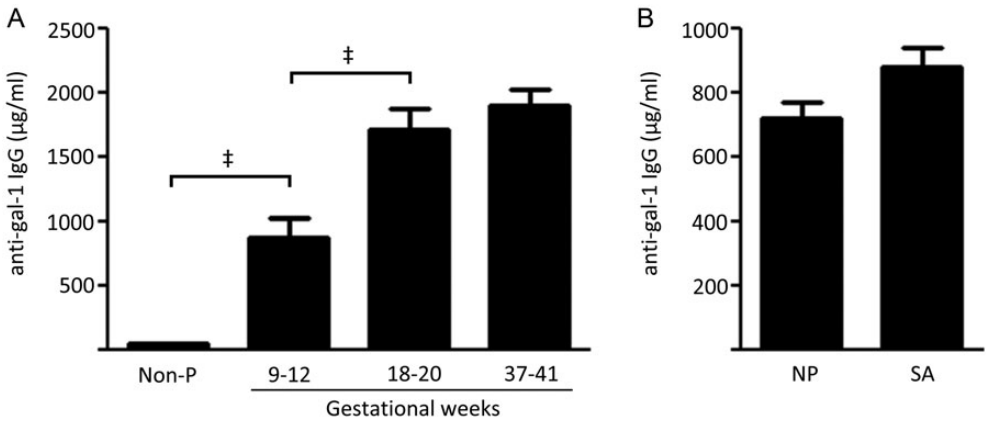

Figure 6 Circulating levels of anti-gal-1 IgG during normal and pathological human pregnancy. (**A**) Systemic anti-gal-1 IgG levels as measured by ELISA. Auto-antibodies anti-gal-1 IgG serum levels from patients with normal pregnancy were increased compared with control non-pregnant (non-P) patients. Results are presented as mean ± SEM ($^{‡}P < 0.001$ as analysed by one-way ANOVA with Tukey's post test. (**B**) Anti-gal-1 IgG serum levels from control patients were similar to those observed pregnant women who subsequently had a miscarriage. NP, normal pregnancy; SA, spontaneous. Results are presented as mean ± SEM and analysed by the Mann–Whitney *U*-test. Characteristics of the participants used in this study are summarized in Tirado-Gonzalez *et al.*, 2013.

who subsequently suffered from spontaneous abortion and those who had a normal pregnancy, similar levels were found in both groups, suggesting that anti-gal-1 auto-antibodies are not involved in the aetiology of spontaneous abortion and their presence does not compromise the course of a normal gestation. However, since anti-gal-1 auto-antibodies have been implicated in other immunological disorders (Xibille-Friedmann *et al.*, 2013), it may be important to determine whether these auto-antibodies detected in the study by Ramhorst *et al.* are a cause or consequence of the RSA. In this context, besides the western blot showing anti-gal-1 reactivity of serum samples, it would have been more precise to provide quantitative data and investigate the correlation between anti-gal-1 and circulating lectin levels in RSA patients.

Pre-eclampsia

Considered a multisystemic maternal syndrome, pre-eclampsia is characterized by proteinuria, hypertension and endothelial dysfunction and is the leading cause of perinatal mortality and morbidity (Lain and Roberts, 2002). Since pre-eclampsia is a syndrome, clinical symptoms could be present at different levels of severity and it is now accepted that pre-eclampsia could be divided in two subtypes depending on the week of delivery. Thus, early onset pre-eclampsia (<34 weeks) represents the severe type, whereas late onset pre-eclampsia (>34 weeks) could be seen as a moderate type of this syndrome (Redman and Sargent, 2005). Interestingly, we have recently found that gal-1 is the only galectin member dysregulated during pre-eclampsia (Freitag *et al.*, 2013). Placental gal-1 expression is decreased in patients who developed early onset pre-eclampsia, whereas this lectin is up-regulated on placental villous tissue in late onset pre-eclampsia patients, compared with those with uneventful pregnancies (Jeschke *et al.*, 2007; Than *et al.*, 2008a, b; Freitag *et al.*, 2013), supporting the hypothesis that the two clinical entities have different aetiologies. Moreover, the frequency of circulating gal-1-expressing NK cells and T cells are reduced in pre-eclampsia patients compared with that in healthy pregnant women, suggesting that gal-1 could be responsible for counteracting the exacerbated maternal immune response observed during the course of pre-eclampsia (Molvarec *et al.*, 2011). Additionally, low gal-1 circulating levels during the second trimester have been associated with the development of PE later on, as demonstrated in a human pilot study (Freitag *et al.*, 2013). Gal-1 may therefore have a potential application as a preclinical biomarker for the development of this syndrome, although this should be tested in a much larger prospective study.

The involvement of gal-1 in the pathogenesis of pre-eclampsia is further supported by *in vivo* studies in mice where the administration of anginex, an artificial β-peptide that specifically inhibits gal-1 pro-angiogenic functions, results in the development of a pre-eclampsia-like syndrome. In this study, we also confirmed that *Lgals1*-deficient mice suffer from pre-eclampsia-like symptoms late in gestation (Freitag *et al.*, 2013). Thus, dysregulation of gal-1 precedes the development of PE, suggesting that this lectin is critical for healthy gestation.

Gestational diabetes mellitus

Metabolic changes during gestation are essential to provide adequate nutrients to the fetus. Affecting up the 18% of pregnancies, gestational diabetes mellitis (GDM) is defined as glucose intolerance with first onset during gestation, increasing maternal and infant morbidity and mortality (Hadar and Hod, 2010). Children born to women with gestational diabetes mellitis suffer from macrosomia and have higher rates of type 2 diabetes and obesity (Malcolm, 2012). Glucose is the primary substrate for energy metabolism in the placenta and fetus. Indeed, between 30–40% of the total amount of glucose taken up by the placenta from the maternal circulation is consumed by the placenta itself. Glucose transport across the placental barrier is facilitated by glucose transporters (GLUT) expressed in the syncytiotrophoblast, being GLUT-1, the primary isoform mediating the transplacental glucose transport during the third trimester (Bissonnette *et al.*, 1981; Jansson *et al.*, 1993). Thus, during the course of GDM, maternal hyperglucemia results in an increased placental glucose transfer which increases fetal insulin secretion and concomitantly enhances fetal growth. Of note, it has been

recently shown that gal-1 up-regulates GLUT-1 expression on mouse embryonic stem cells (Lee and Han, 2008). Therefore, it is logical to assume that gal-1 could regulate glucose metabolism also in the placenta. However, it is still unknown whether gal-1 is differentially expressed during the course of GDM and more importantly, if the circulating levels of this lectin are deregulated in women who subsequently suffer from GDM. Furthermore, it would be interesting to analyse the interplay between pre-eclampsia and diabetes in the context of gal-1 deregulation. For instance, it has been shown that women with pre-eclampsia have an increased risk of developing type 2 diabetes after pregnancy (Feig *et al.*, 2013) and presence of pre-eclampsia in the setting of GDM also raises the risk of type 2 diabetes beyond that seen with GDM alone.

Galectin-1 in malignancies of female reproductive tissues

Altered expression of gal-1 is frequently associated with cancer and malignancies of different female reproductive organs are no exception to this.

Gestational trophoblastic disease

Gestational trophoblastic disease comprises medical conditions with an abnormal trophoblast cell differentiation and proliferation leading to partial or complete hydatiform moles with malignant transformations like invasive moles, choriocarcinoma and placental site trophoblastic tumours in some cases. Elevated gal-1 levels in first trimester invasive transformed trophoblasts, characterized by a disorganized and invasive growth, were demonstrated in patients with invasive moles and choriocarcinomas (Bozic *et al.*, 2004). Whether the increase in gal-1 is a consequence or a cause of this disease remains unclear. On the one hand, gal-1 is able to promote cell migration and transformation and the invasion of EVTs (Paz *et al.*, 2001; Camby *et al.*, 2006; Kolundzic *et al.*, 2011) so that the increased gal-1 expression may cause the transformed phenotype and disorganized invasion of trophoblasts. On the other hand, cell transformation is often accompanied by a deregulation of gene expression, which may also account for the increase in gal-1 expression. Moreover, gal-1 seems to be implicated in tumorigenesis and metastasis of melanomas and gliobastomas by promoting tumour cell adhesion, migration and angiogenesis and escaping the immune system (Braeuer *et al.*, 2012).

Ovarian cancer

Using a proteomic approach, Chow *et al.* (2010) identified gal-1 as one of the proteins that is elevated in ovarian cancer when compared with normal tissues. This was confirmed by Kim *et al.* who assessed gal-1 expression in epithelial ovarian cancer patients by immunohistochemistry and observed no staining in normal tissue while all cancer tissues stained positive. Furthermore, high expression of gal-1 in peritumoural stroma was associated with poor progression free survival (Kim *et al.*, 2012). Increased stromal expression of gal-1 in epithelial ovarian carcinomas when compared with normal non-invaded stroma was also reported by van den Brule *et al.* (2003). The latter study also showed that gal-1 was expressed and secreted by several ovarian carcinoma cell lines, although Fredriksson *et al.* (2008) did not observe differences in gal-1 plasma levels between ovarian cancer patients and healthy controls. This is somewhat surprising since gal-1 binds to CA125, the ovarian cancer antigen that is widely used to monitor patients with epithelial ovarian cancer (Karam and Karlan, 2010; Seelenmeyer *et al.*, 2003). Nevertheless, *in vitro* analyses have shown that exogenous gal-1 can induce proliferation in ovarian cancer cell lines, enhance adhesion to different substrates and increase the invasive capacity of tumour cells (Woynarowska *et al.*, 1994; van den Brule *et al.*, 2003; Kim *et al.*, 2012). Additionally, MUC1 is overexpressed in ovarian carcinoma and since gal-1 binds MUC1 via the TF antigen, there might be an involvement of gal-1 (Fischer *et al.*, 2009; Engelstaedter *et al.*, 2012). Gal-1 is also expressed by the human choriocarcinoma cell lines JEG-3 and BeWo (Vicovac *et al.*, 1998).

Overall, these findings link increased ovarian gal-1 expression to enhanced tumour aggressiveness and poor patient outcome. Consequently, patients might benefit from gal-1 targeted therapy, which is supported by preclinical observations using *in vitro* and *in vivo* models of ovarian cancer (Woynarowska *et al.*, 1994; Dings *et al.*, 2010; Dings *et al.*, 2013).

Uterine cancer

Reports on gal-1 expression in uterine cancers are scarce and inconclusive. van den Brule *et al.* (1996) described increased gal-1 expression in advanced adenocarcinoma cells when compared with normal endometrium. Using biotinylated gal-1 and subsequent staining, Mylonas *et al.* (2007) found that gal-1 binding increased with increasing grade of endometrioid adenocarcinomas. In addition, gal-1 binding correlated with lymphangiogenesis, which has been associated with poor prognosis. While these data suggest that gal-1 levels increase during uterine cancer progression, another study did not see any difference in gal-1 expression or binding when comparing uterine leiosarcomas with leiomyomas (Schwarz *et al.*, 1999). However more recently, Weissenbacher *et al.* (2011) did observe increased gal-1 levels in myoma when compared with both normal myometrium and leiomyosarcoma. Most likely, gal-1 expression is dependent on the localization, stage and type of uterine cancer and more studies are required to gain a better insight into the relationship between gal-1 expression and uterine tumour progression.

Cervical cancer

Like cancers of the uterus, limited information is available regarding the expression of gal-1 in cervical cancer. Only recently, Kim *et al.* (2013) reported that gal-1 is exclusively expressed in human cervical cancer tissues but not in normal cervical epithelium. Furthermore, gal-1 was frequently expressed in the peritumoural stroma, which is comparable to their observations in ovarian cancer tissues (Kim *et al.*, 2013). This is also in agreement with Kohrenhagen *et al.* (2006) who found that stromal gal-1 expression was positively associated with the histopathological grade of cervical cancer tissues. Both studies indicate a relation between stromal gal-1 expression and tumour progression. Indeed, Kim *et al.* found that increased gal-1 expression was associated with depth of invasion and a positive lymph node status. Further *in vitro* studies confirmed that overexpression of gal-1 enhanced the proliferation and invasiveness of cervical tumour cells. A study by Li *et al.* linked gal-1 expression by cervical tumour cells to enhanced immune suppression, since treatment with gal-1 antibody increased the inhibitory effect of tumour infiltrating lymphocytes on xenograft tumour growth in SCID mice (Li *et al.*, 2010). Despite all these findings, the study by Kim *et al.* (2013) did not find a difference in the 5-year progression free survival between patients with high or low gal-1 expression.

Breast cancer

Of all organs related to female reproduction, breast is the best studied with regard to gal-1 expression. In a galectin profiling study, gal-1 was found to be expressed by all breast cancer cell lines tested (Lahm *et al*., 2001). Microarray analysis comparing breast cancer cell lines with either low or high ERBB2 (HER2/neu) expression identified gal-1 as the most up-regulated gene in the latter group (Mackay *et al*., 2003). André *et al*. (1999) found that increased gal-1 binding was associatedwith positive lymph node status as well as with the tumour size and stage of breast cancer patients. Supporting observations were made more recently by Dalotto-Moreno *et al*. who found increased gal-1 expression in tumour cells and tumour stroma of breast adenocarcinomas when compared with benign breast hyperplasia. Furthermore, they described that increasing numbers of gal-1-positive cancer cells were positively associated with the histological grade (Dalotto-Moreno *et al*., 2013). Finally, several studies employing different proteomic approaches also point towards the involvement of gal-1 in breast cancer, even in males (Kreunin *et al*., 2007; Chahed *et al*., 2008; Imai *et al*., 2008; Gromov *et al*., 2010; Xu *et al*., 2010).

Given that increased gal-1 expression is associated with breast cancer progression, it appears surprising that Barrow *et al*. (2011) found that circulating gal-1 levels were significantly decreased in breast cancer patients when compared with healthy controls. In addition, there was no relation between serum gal-1 levels and mortality risk (Barrow *et al*., 2011). This is even more remarkable given the observation that the serum of breast cancer patients does contain more gal-1-binding glycoproteins (Carlsson *et al*., 2011; Carlsson *et al*., 2012). Apparently, the increased levels of gal-1 stay confined to the tumour tissue where the protein exerts its function. On the other hand, gal-1 can be subjected to cleavage by different MMPs that are expressed by breast cancer cells (Overall and Dean, 2006; Butler *et al*., 2008; Kleifeld *et al*., 2010). Possibly, these gal-1 proteolytic fragments are no longer detectable in the serum. Furthermore, proteolytic processing of gal-1 could provide some insight in the function of the protein during breast cancer progression. For example, local degradation of gal-1 by MMPs might hamper homotypic tumour cell aggregation thereby facilitating tissue extravasation and metastasis. On the other hand, Glinsky *et al*. (2000) observed increased clustering of gal-1 in MDA-MB-435 breast cancer cells at sites of interaction with endothelial cells. This suggests that the cells require gal-1 for heterotypic cell adhesion at distant sites of metastasis. In line with this, several studies link gal-1 expression to the metastatic potential of breast cancer cells (Kreunin *et al*., 2007; Imai *et al*., 2008; Xu *et al*., 2010). Furthermore, blocking of gal-1 hampers metastasis formation by metastatic breast cancer cells in mice and this was accompanied by a reduction in immunosuppression (Dalotto-Moreno *et al*., 2013). A reversal of immunosuppression upon gal-1 targeting was also shown by Stannard *et al*. (2010) in a murine breast tumour model. Thus, gal-1 targeted therapy could be beneficial for breast cancer patients on multiple levels, including the potential anti-angiogenic effects as described earlier.

Concluding remarks

Despite the significant results obtained and the efforts made during the last decade, research into the functions of gal-1 is in its early stages. This review provides a solid base for the role of gal-1 in maternal fetal tolerance, regulation of growth, differentiation, invasion and immune evasion mechanisms of trophoblast cells during placentation. We also put great emphasis on the interplay of gal-1 and hormones (e.g. progesterone) during pregnancy. This review also addresses the gal-1-mediated angiogenesis during gestation and the involvement of gal-1 as a potential biomarker to predict pregnancy outcome.

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Authors' roles

All authors were involved in preparing this manuscript. G.B., N.F. and S.M.B. were the main contributors to the description, the immune and pro-angiogenic functions of gal-1 and the reproductive disorders influenced by gal-1. I.T.-G. performed and analysed the work of autoantibodies against gal-1. L.U. and U.J. contributed to the section about intracellular signalling pathways activated by gal-1. V.L.J.L.T. contributed with the information on gal-1 in malignancies of female reproductive tissues. S.M.B. conceived and designed this review article.

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Conflict of interest

The authors declare no conflict of interest.

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