Roles of galectins in vivo

Françoise Poirier

Institut Jacques Monod, 2 Place Jussieu, 75251 Paris cedex 05, France

Abstract

The mutational analysis of the galectin family is shedding a different light of this class of molecules. On the one hand, it appears that galectin 1 and galectin 3 are not required for the survival of mice in normal animal house conditions, while on the other hand, there seems to be several subtle, but very complex, consequences of lacking galectins during development.

Introduction

Galectins, or S-type lectins, are a family of carbohydrates-recognition proteins that are defined by their shared structure and their affinity for β-galactoside-containing glycoconjugates [1–3]. Galectins were originally isolated from mammalian tissues, but they have now been found in many other species including birds, fish, worms and sponges [4]. Twelve different mammalian galectins have been identified to date. Despite the absence of signal peptides, galectins can be secreted outside the cells by a novel pathway independent of the Golgi apparatus [5]. Members of the galectin family have been found in all subcellular compartments (extracellular, cytoplasmic and nuclear) in vivo, depending on the tissue type and the stage of the cell cycle. The subcellular localization is likely to be a crucial parameter in the understanding of the function fulfilled by a given galectin at a given time.

Most of the work on galectins concerns their potential roles in tumour biology and inflammation [6,7]. In addition, galectins have been studied in many in vitro tissue culture systems, but no simple unifying theory is emerging from this wealth of data. Instead, several different functions have been assigned to galectins, ranging from structural roles to signal transmission; they have been described as modulators of cell adhesion [8–11], regulators of cell survival [12,13] and even as splicing factors [14].

With the goal of identifying developmental processes in which galectins participate in vivo, our laboratory has initiated a genetic analysis of the galectin gene family in the mouse. Mouse lines carrying deletions of the galectin 1 or

1 E-mail poirier@ijm.jussieu.fr
galectin 3 genes have been generated. Because both mutations are viable and fertile, these animal models provide good tools to unravel the various roles of this class of molecules in vivo.

**Different galectins are expressed in different tissues during development**

We first examined the distribution of galectin gene transcripts by in situ hybridization and, in some cases, by antibody staining on whole mouse embryo sections. This analysis has thus far been carried out for galectins 1, 3, 4/6 and 7 (Figure 1). All these genes are expressed during development, and each of them displays a restricted and unique expression pattern. These characteristics are also shared by galectin 5 even though it has not yet been characterized as extensively. Galectin 1 first appears in the trophoderm (extra-embryonic) cells of the blastocyst at the time of implantation (E4.5, day 4.5 of embryogenesis), and starting from E9.5, galectin 1 becomes broadly distributed in the embryo proper, although this distribution is restricted mostly to tissues of mesodermic origin [15].

Galectin 3 is also first detected in the trophoderm cells at E4.5. Within the embryo, its expression is confined almost entirely to developing bone tissues until midgestation. At later stages, galectin 3 transcripts are also found in various epithelial tissues, including kidney, bladder, intestine and skin [16]. In addition, galectin 3, which is also known as Mac2 [17], is expressed in the macrophage lineage.

Galectin 4 and galectin 6 are both found exclusively in the epithelial cells of the developing intestine [18]; however, given the extensive degree of sequence similarity between galectin 4 and galectin 6, the in situ results had to be corroborated by RNase protection experiments. Finally, galectin 7 expression is restricted to stratified epithelia, regardless of their state of keratinization or their regional specialization. Therefore, the main galectin 7-expressing tissue is the epidermis, but it is also found in the oesophagus, tongue, lip, cornea and Sertoli cells [19].

Several conclusions can be drawn from these observations: (i) all tested galectins are expressed in the developing embryo; (ii) each appears to be involved in specific developmental processes, e.g. galectin 3 in bone formation, galectins 4 and 6 in intestine morphogenesis, and galectin 7 in epithelial stratification; (iii) apart from galectins 4 and 6, which clearly result from a recent gene duplication [20], so far, no two galectins are expressed in the same way. Although galectin 1 and galectin 3 transcripts, for example, are simultaneously present in the trophoderm cells of the implanting embryo, the corresponding proteins have strikingly different subcellular localization; galectin 1 is found mainly in the cytoplasm, while galectin 3 is nucleus- and membrane-associated [21]. Similarly, galectins 3 and 7, are co-expressed in the epidermis, but galectin 3 behaves like all other suprabasal differentiation markers, whereas galectin 7 displays a totally novel expression pattern, in the sense that it is barely detectable when there is a single layer of suprabasal cells and is very strongly expressed when there are several layers of suprabasal cells.
In summary, a refined and dynamic repertoire of galectins is set up, starting early during mouse embryogenesis. In no case have the corresponding physiological glycosidic ligands been identified, but one can imagine that an equally refined and dynamic repertoire of glycosyltransferases is also established at the same time, presumably generating an immensely complex glycosidic ‘map’ of the embryo. Thus, a whole range of galectin–glycoside interactions might participate in many morphogenetic processes that take place during development. The identification of Fringe, a modulator of Notch, as a glycosyltransferase represents an example of how a specific glycosylation event may regulate developmental processes [22].

**Galectins and embryonic implantation**

We originally isolated galectin 1 in a differential screen that was designed to identify genes regulated at the time of embryonic implantation [23] and indeed the activation of galectin 1 expression in trophectoderm cells is concomitant with hatching of the blastocyst out of the zona pellucida (which is the ultimate step before attachment). This suggests a role for this lectin in the initial interactions between the embryo and the uterine wall [15]. Although the molecular events that mediate embryonic implantation remain to be elucidated, it has been well documented that carbohydrate residues located on the surface of the maternal tissue are involved in this key process of mammalian development [24]. However, when a null mutation was introduced into the mouse galectin 1 gene, it was found to be compatible with fertility and survival in animal house conditions [25]. Interestingly, the only other gene known to follow the same distinct activation pattern in E4.5 embryos is galectin 3. By pursuing our genetic approach, we found that a galectin 3 null mutation was also compatible with fertility and survival, and that even galectin 1/galectin 3 double mutants exhibit no overt phenotype [21]. This may not be too surprising since, as mentioned above, the two proteins have different subcellular localizations, even in the implanting embryo.
It remains formally possible that galectin 1 and/or galectin 3 normally participate in the process of implantation and that a backup mechanism operates in the mutants. This idea is supported indirectly by the fact that galectin 1 can accelerate the rate of blastocyst attachment on to endometrial cells \textit{in vitro} (S. Kimber and F. Poirier, unpublished work). Alternatively, this crucial step of mammalian embryogenesis may be secured by several systems, all of which function normally and in parallel. If this is the case, the absence of one system (i.e. galectin–carbohydrate interactions) may have a very limited effect; for example, a short delay in the timing of implantation is conceivable.

\textbf{Mutant phenotypes in gal-1$^{-/-}$ mice}

The existence of mice that are homozygous galectin 1 mutants (gal-1$^{-/-}$ mice) have allowed us to start testing various hypotheses \textit{in vivo} regarding galectin functions drawn from \textit{in vitro} data.

A subtle, but robust, phenotype was discovered in the developing olfactory system. In the absence of galectin 1, a subpopulation of primary olfactory neurons fails to project to their correct target sites in the caudal olfactory bulb [26]. Thus, galectin 1, which is normally expressed transiently by ensheathing cells of the olfactory nerve fibre at E15.5 [27], appears to be involved in the guidance, growth or maintenance of primary sensory olfactory axons between the nasal cavity and the olfactory bulb. This was the first evidence that a lectin has neurite outgrowth-promoting activity and plays a role in neuronal pathfinding in the mammalian nervous system. Potential functional consequences of this defect remain to be explored.

Galectin 1 had long been identified as an immunomodulating factor [28]. Most importantly, galectin 1 can induce apoptosis when added exogenously to human T-cells in culture [12]. Surprisingly, histological examination of the thymus and spleens from the gal-1$^{-/-}$ mice revealed no overt differences compared with wild-type mice. In the thymus of gal-1$^{-/-}$ mice, cortical and medullary regions were of similar size to those seen in the thymus from wild-type mice, with sharp corticomedullary junctions. In spleens from gal-1$^{-/-}$ mice, well-developed white pulp was present, and the number of lymphoid follicles did not differ from that observed in spleens of wild-type mice (L. Baum, personal communication).

Finally, it has been reported that galectin 1 is specifically induced in activated T-cells [29] and it is able to inhibit antigen-induced proliferation of naive and memory CD8$^{+}$ T-cells \textit{in vitro}. It was therefore proposed as an autocrine negative growth factor [29]; however, the galectin 1 null mutant mice were indistinguishable from wild-type littersmates with respect to lymphocyte distribution and cytolytic T-lymphocyte activity after lymphocytic choriomeningitis virus infection (H. Pircher, personal communication).
Mutant phenotypes in gal-3\(^{-/-}\) mice

Galectin 3 has also been implicated in the immune system, especially in inflammatory responses. Expression of galectin 3 has been described on neutrophils [30], eosinophils [31] and also on activated macrophages (Mac-2 marker) [17]. Therefore, as in the case of galectin 1, several studies focused on the immune system of galectin 3 mutants. The first set of experiments was to examine the response induced after triggering acute peritonitis by intraperitoneal injection of thioglycolate. We observed that recruitment of mutant granulocytes at the site of inflammation was normal, but that their maintenance inside the peritoneal cavity was impaired. Since neither massive apoptosis nor any major increase in macrophage phagocytic activity were detected, these results suggested the existence of an additional level of control during the resolution of acute inflammation [32]. It seems likely that galectin 3, which is normally present on the surface of granulocytes, participates in an active retention mechanism and prevents the cells from leaving the site of inflammation. Using similar experimental conditions, Hsu et al. [33] noticed an abnormal behaviour of galectin 3 mutant macrophages, suggesting another role for galectin 3 in macrophage cell shape, migration and possibly survival.

Several developmental aspects of the galectin 3 phenotype are under investigation, but the most extensive and informative study to date has been carried out in developing long bones because the galectin 3 gene is normally expressed from the earliest stage of bone formation (Figure 1). Adult galectin 3 mutants are of normal size and seem to have normal bones; however, there are many well-documented examples of major defects in bone development which get corrected at later stages. We therefore undertook a thorough analysis of the process of ossification in the absence of galectin 3.

We found that mutant long bones do indeed exhibit multiple, though subtle, defects during embryogenesis, both in the process of chondrogenesis and in the co-ordination between chondrogenesis and osteogenesis [34] (Figure 2). Several aspects of chondrogenesis are affected: abnormal glycogen aggregates appear in proliferating and maturing mutant chondrocytes, the synchrony of chondrocyte differentiation along the longitudinal axis of the bone is altered and, most importantly, the final stages of chondrocyte differentiation are accelerated as revealed by a size reduction of the terminal hypertrophic zone (Figures 3A–3D) and a higher incidence of ‘dark’ cells, i.e. cells with dense cytoplasm and nuclei containing finely condensed chromatin and large vacuoles. This latter point is particularly important because we have shown that these ‘dark’ cells correspond to the final step before chondrocyte death; however, these cells are not apoptotic, either by histological criteria (they contain many cytoplasmic organelles and no apoptotic bodies) or by the terminal transferase deoxytidyl uridine end-labelling assay. Taken together with recent results from a study of the rabbit growth plate [35], the study of the defects in long bones from gal-3\(^{-/-}\) mice establishes that chondrocytes do not die by apoptosis. Instead, they follow an alternative pathway of programmed cell death, more similar to type II or lysosomal cell death as has been described during insect metamorphosis [36,37] or ovarian atrophy [38].
In addition, the various defects of chondrogenesis detected in the absence of galectin 3 also include limited and abnormal mineralization of the cartilage matrix, as well as sparse, short and irregular collagen fibres in the extracellular matrix, as revealed by electron microscopy (Figures 3E and 3F).

The most visible defect in the growth plate of long bones of gal-3\textsuperscript{-/-} mice is the presence of empty lacunae at the junction between the last row of hypertrophic chondrocytes and the front of vascular invasion (Figures 3A–3D). This is a unique feature that is characteristic of gal-3\textsuperscript{-/-} mutants and these results show, for the first time, that the processes of chondrogenesis and osteogenesis can be uncoupled. In other words, the triggering of programmed cell death in hypertrophic chondrocytes does not require direct contact with osteoclasts, osteoblasts or blood cells.

In conclusion, the panel of defects observed in growth plates from gal-3\textsuperscript{-/-} mice provides new fundamental information that contributes to the understanding of the normal process of bone development. An important aspect of this study is the complexity of the mutant phenotype that, even within the bone, cannot \textit{a priori} be explained by a single unique function of galectin 3. Rather, the range of defects implies that galectin 3 fulfils several functions during endochondral ossification, notably as a survival factor for hypertrophic chondrocytes and as a molecule that mediates, directly or indirectly, the coordination between chondrogenesis and osteogenesis. Although the precise molecular events underlying this phenotype remain to be elucidated, a systematic deregulation of Indian hedgehog expression in the bones of gal-3\textsuperscript{-/-} mice has been documented, which suggests that the absence of galectin 3 somehow impinges on the control of this key regulator of chondrogenesis [39].
Conclusions and perspectives

The mutational analysis of the galectin family is now shedding a different light on this class of molecules. On the one hand, it appears that galectins are not required for survival in animal-house conditions; on the other hand, there seem to be many subtle, but very complex, consequences of lacking galectins.

**Figure 3** Histological comparison of wild-type and galectin 3 mutant embryonic femurs. Optical semi-thin sections (1 μm) from wild-type (A and C) and mutant (B and D) femurs were photographed under low (A and B) and high (C and D) magnification. Note the gap between the last row of hypertrophic chondrocytes and the vascular front in the mutant. Electron-microscopic sections from hypertrophic zones of wild-type and mutant mice are shown in (E) and (F) respectively. Zones of sparse and irregular collagen fibres are observed in the mutant.
during development. A thorough description of the galectin null phenotypes may require extensive examination, including electron microscopy studies. For instance, just as bone development is abnormal in galectin 3 mutants, even though the adult bones appear normal, so too the process of embryonic implantation might be abnormal in the absence of galectin 1 and/or galectin 3, even though the implantation succeeds in the end. Several interpretations may explain a large panel of mild defects. A possibility remains that strict redundancy is taking place between different members of the galectin family, although we do not have any indication supporting this hypothesis. In this case, the prediction would be that compound galectin mutants would exhibit more defects than the sum of individual mutants. Another, perhaps more attractive, possibility is that parallel systems operate in many developmental processes so that the lack of a galectin can be compensated for by the deregulation of one or several parallel systems. Such separate parallel systems, which are yet to be identified, would presumably be different depending on the biological process (i.e. implantation, olfaction or ossification).

At this stage of the analysis, the emerging picture is that in normal conditions galectins function as ‘optimizing’ molecules, i.e. once removed, the system still works only slightly less efficiently. This has no major consequences for the individual, but it may have some for the population in the long run. In addition, it is now important to evaluate the response of galectin mutants to stress or pathological conditions; for example, the lack of galectin 3 in developing bones might become detrimental for the animal in the case of a vitamin-D-deficient diet, or during the repair mechanism following a fracture.

In view of the complexity of galectin phenotypes, the precise definition of the cell type, subcellular compartment and stage of differentiation when a galectin operates in vivo will be an important step towards isolating their molecular partners in the cell.

I thank all past and present members of the laboratory. This work is supported by the CNRS (Centre National de la Recherche Scientifique) and by the ARC (Association de la Recherche sur le Cancer).

References