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Expression of galectins in cancer: A critical review

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A large body of literature has examined and described galectin expression in cancer. Discrepancies have been observed in the reported data, which hampered clear understanding of the expression profiles. This relates to the use of different types of methods that evaluate either global or specific gene expression in heterogeneous cancer tissue samples, type of antibodies used in immunohistochemistry and procedures of comparison of gene expression. In this manuscript, we review the main data concerning expression of galectins in human cancer. Only galectin-1 and galectin-3, the most abundant and examined galectins, will be examined here.
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Introduction

A major goal in cancer research is to evaluate the potential implication of identified genes and gene products in the biology of the cancer cell. Beside *in vitro* and *in vivo* function studies, one way of achieving this goal is to examine gene expression in cancer lesions. Differential expression of the specifically examined gene in the cancer cells compared to the corresponding normal tissue generally indicates the potential importance of the specific gene for cancer cell biology. Moreover, potential correlation between the parameter examined and progression of the disease should be studied, if possible. This could indicate additional potential prognostic value for the examined parameter. Finally, the potential implication of the examined gene product could lead to the definition of the roles played by the protein, and maybe drive new therapeutic strategies.

In this review article, we examine all published data about galectin expression in cancer. As most data (and contradictions) arise from the two most abundant and studied galectins, galectin-1 and galectin-3, only these two lectins will be examined here.

Criteria for evaluating gene and protein expression in cancer

A large body of reported data about galectin expression in cancer is available in the literature. However, the strength of these published studies is variable, and the conclusions drawn are of different values. This can be related to the study of different type of tissues of cell lines, using numerous techniques that offer several types of advantages and disadvantages.

Indeed, gene expression in cancer can be examined in *in vitro* models (cell lines), *in vivo* models (*e.g.* inoculation of cell lines to nude mice, or induction of tumors by chemicals, and analysis of the primary tumors), and in spontaneous animal or human malignant tumors. In the case of examination of neoplastic tissues, techniques evaluating global gene or protein expression such as Northern and Western blotting, and reverse transcriptase-PCR amplification, should be avoided in most cases because of the heterogeneity of the tissues constituting a strong bias for the expected results. Indeed, the proportion of the considered cells (*i.e.*, including normal epithelial, preneoplastic, neoplastic, stromal, vascular and inflammatory cells) vary in normal and cancerous samples, and contributes to eventually observed modulation of expression. This can explain why mRNA expression levels of cancer cells can be at least masked by the levels of the mRNA present in noncancerous cells. Of course, examination of gene expression should always be cell type-specific, in order to give best insights into tumor cell biology. Moreover, the stromal compartment (associated or not with invading cancer cells) is another source of "dilution" of the examined protein from the cancer cells when using Western blotting. Then, histological techniques such as immunohistochemistry or *in situ* hybridization should be preferred, except for very homogenous samples.

Specific parameters could affect the specificity of the techniques used, including immunohistochemistry. For instance, tissue fixation can affect antigen detection. We always used in our studies formalin-fixed, paraffin-embedded tissues samples, including for assessment of antibody specificity. In cases where

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an antibody does not allow antigen detection in immunohistochemistry (and, for instance, with specific band detected by Western blotting), antigen retrieval techniques could be used. In the case of galectin-1 and galectin-3 detection, no such techniques were used in our experiments, as reactivity has always been observed as expected in formalin-fixed tissues, with satisfactory cell line-based specificity [1,2] and adsorption experiments [3–5].

In this review, we will only consider here the studies concerning human malignant tumors, with clear methodology allowing to draw firm conclusions about galectin expression.

Galectin-1

Galectin-1 has been the subject of numerous expression studies. Different expression profiles have been detected for cancer cells and the surrounding host tissues, such as in breast carcinoma [6]. Heterogenous expression was detected by Western blotting and by immunohistochemistry in a series of colon carcinoma samples, without reported modulation between normal and tumor cells [7]. An other study on a series of 46 colon carcinoma reported a localisation of galectin-1 in the stroma, and no modulation of the lectin in the examined samples using Western blotting [8], which can be expected from tumor heterogeneity. The same observation was made using immunohistochemistry in head and neck squamous cell carcinoma [9]. An other study demonstrated immunohistochemical galectin-1 expression in ovary cancer cells present in effusions [10,11]. A study based on global Northern blotting experiments demonstrates increased galectin-1 mRNA levels in high-grade bladder carcinomas compared with low-grade tumors and normal bladder [12], and in gliomas [13].

Increased expression in cancer cells compared to normal cells has been demonstrated in several tumor types. Several studies demonstrated increased expression in cancer cells including from bladder [12] and thyroid [14–16] compared to normal cells, by Northern and Western blotting and immunohistochemistry. Immunohistochemistry experiments performed in our laboratory demonstrated increased galectin-1 expression in endometrial adenocarcinoma cells compared to the corresponding normal cells [2]. Strong galectin-1 immunopositivity of glioma cells was correlated to short survival [17]. To date, a single study demonstrated that galectin-1 expression is decreased, in head and neck cancers [18].

Other studies highlighted the stromal expression of galectin-1, associated to cancer cells, and suggesting induction of a host tissue response. Immunohistochemistry demonstrated that galectin-1 was detected in the stroma around the negative head and neck squamous cell carcinoma [9]. A well-conducted immunohistochemical study including adequate controls for antibody specificity demonstrated localization of galectin-1 in stromal cells but not in epithelial cells; galectin-1 expression in the tumor-associated stroma was correlated with progression from normal to precancerous and cancerous tissues [3]. Galectin-1 expression was also demonstrated in the stroma invaded by pancreatic cancer by immunohistochemistry and *in situ* hybridization [19]. Increased galectin-1 expression was observed in intrahepatic cholangiocarcinoma cells as well as in associated stroma [20].

Galectin-1 expression in prostate carcinoma has been examined in several studies. A first report demonstrated that galectin-1 is expressed in a collection of 7 normal, 8 prostatic intraepithelial neoplasia, 20 primary adenocarcinomas and 12 metastases [21]. Using adequate specificity controls for the polyclonal antibody used, we demonstrated recently that prostate cancer cells, as well as normal and PIN cells, do not express galectin-1; preferential expression of galectin-1 in cancer cell-associated stroma is observed in 21.3% of the examined cases and is an independent predictor of prostate specific antigen (PSA) recurrence in multivariate statistical analysis [5]. This suggests that induction of galectin-1 expression in host stromal cells by prostate carcinoma cells can modulate tumor progression, and influence disease outcome.

Similar observations, *i.e.* absence of galectin-1 immunoreactivity in normal and cancer cells and noninvaded stroma, and strong expression in the cancer cell-associated stroma, were observed in pancreatic cancers, although the small population size did not allow to observe correlation of these parameters to disease progression [19].

Another interesting observation is that we observed an increased frequency of galectin-1 immunostaining in prostate cancer-associated capillaries than in those from non invaded stroma [22]. This observation was correlated to *in vitro* data demonstrating that conditioned medium from prostate cancer cells increased galectin-1 expression in HUVEC and heterotypic cell-cell adhesion of PC-3 prostate cancer cells to pretreated HUVEC monolayer [22].

These observations suggest that galectin-1 expressed by cancer cells could contribute to the invasive phenotype of the cancer cells, such as during cancer cell adhesion to the matrix and cell-cell aggregation. Moreover, soluble(s) factor(s) released by the cancer cells could induce galectin-1 expression and release by stromal fibroblasts. Stromal galectin-1 could also influence cancer cell biology and induce apoptosis of activated lymphocytes [23], constituting a shield decreasing antitumoral immunity. The mechanisms of galectin-1 accumulation in cancer-associated stroma, and its consequences for cancer biology remain to be explored.

Galectin-3

A large body of published data is available in the literature. Conflicting results have been published, resulting in difficulties to draw a clear picture of galectin-3 expression profiles in cancer.

Initial studies suggested that galectin-3 expression was increased in cancer cells in correlation with their invasive potential. These data were based on *in vitro* models using cancer cell lines [24–26] and human colon cancer specimens [7,8,27]. These studies used immunohistochemistry and Western blotting

analyses on total tumor protein extracts. Most immunohistochemical experiments used the monoclonal antibody M3/38, that was raised against glycoproteic extracts obtained by lectin affinity chromatography from membrane fractions of thioglycolate-elicited mouse macrophages that were previously immunodepleted from previously known antigens [28]. The recognized epitope is thought to be situated on the N-terminal domain of galectin-3, in the repeating domain (Qian and Leffler, unpublished). Although it detects a unique band in Western blotting, there are no published data that verify the monospecificity of M3/38 in immunohistochemistry. Although difficult to control, it is not possible to exclude that the use of M3/38 in immunohistochemistry provides cross-reactions with other known or unknown molecules.

Increased expression in cancer cells (as detected with M3/38) was also observed in cancers from thyroid [16,29–35], central nervous system [36], head and neck squamous cell carcinoma [9], pancreas [37], bladder [12], stomach [38] and renal carcinoma [39].

Increased galectin-3 expression in the primary colon cancer cells was correlated to decreased survival [40] or decreased disease-free survival [41], suggesting a prognostic value for the detection of galectin-3 expression.

Interestingly, increased expression of galectin-3 was also observed in hepatocellular cancer cells using either a polyclonal antibody or the monoclonal anti-recombinant galectin-3 A3A12 antibody [42].

We have conducted several studies using immunohistochemistry and an anti-galectin-3 polyclonal antibody [43]. Immunostaining specificity was controlled by examining cancer cell pellets that express or not galectin-3, that were fixed in buffered formalin and embedded in paraffin [1]. We also used adsorption experiments in which the immunostaining activity of the polyclonal antibody was inhibited by preincubation with recombinant galectin-3 [3,4].

Initial experiments demonstrated that galectin-3 global expression was decreased in colon cancer specimens compared to corresponding normal tissue, as determined by Northern and Western blotting [44]. Immunohistochemistry confirmed this expression profile in cancer from ovary [45], breast [1], endometrium [2], colon [2] and skin [46]. These results were in apparent opposition with the previously described results, and were the subject of many discussions.

Other groups have obtained similar results as ours, either with polyclonal antibodies, *e.g.* in skin [47], head and neck [18] and prostate cancer [48], or with M3/38 in cancers from colon [49], prostate [21], salivary glands [50] or intrahepatic cholangiocarcinoma [20]. These latter results suggest a crucial influence of staining evaluation methods in these studies. It is also possible that antibody crossreactions could be present with specific tissues and not in others, explaining diverging results.

However, immunohistochemistry experiments using a polyclonal antibody demonstrated increased galectin-3 expression in thyroid cancer, compared to normal cells [51]. Another study using a polyclonal anti-galectin-3 antibody demonstrated increased galectin-3 expression in pancreatic cancer cells compared to corresponding normal cells [19].

Some studies demonstrated combined profiles. For instance, astrocytic tumors show decreased galectin-3 expression, but a small fraction of tumors expressing high levels of galectin-3 were characterized by increased aggressiveness [52].

Several authors have suggested that detection of galectin-3 could help to better define diagnosis and/or prognosis of cancer. For instance, increased galectin-3 expression in cancer tissue compared to the adjacent normal tissue or benign lesions was demonstrated for thyroid papillary carcinomas [16,29,30,32–35,51,53–59]. In the last case, the diagnostic utility of galectin-3, alone [54,60–62] or with other markers [63–65], has been recently abundantly reported. Overexpression of galectin-3 mRNA in papillary thyroid cancer was determined by quantitative RT-PCR [66]. However, in contrast to all these published data, a new study has reported that galectin-3 mRNA and protein are also expressed in benign thyroid tumors, suggesting caution when using galectin-3 as a diagnostic marker [67]. Another limitation has been reported when studying galectin-3 mRNA expression in fine-needle aspirates by RT-PCR: Hashimoto thyroiditis was also scored positive [68]. Immunohistochemical detection of galectin-3 was also shown in a significant number of nonneoplastic cases [69]. Thus, galectin-3 could be recommended for help in diagnosis of thyroid cancer [70,71], although it is not an universal and perfect marker of this malignancy.

Several observational and functional studies had demonstrated that galectin-3 could be shuttled to the nucleus of fibroblasts [72–76], be a component of ribonucleoproteic complex [77], bind DNA [78] and play a role during mRNA splicing [79]. Thus, the attention of researchers was attracted by cellular localization of galectin-3. Normal cells from breast, endometrium, colon and prostate are characterized by nuclear and cytoplasmic galectin-3 cytolocalization. We have established a correlation between the exclusive cytoplasmic localization of galectin-3 and aggressiveness of cancer cells from endometrium (assessed by the depth of myometrial invasion) [2], colon (assessed by low survival) [3] and prostate (assessed by PSA increase) [4], using a polyclonal antibody. Increased cytoplasmic galectin-3 expression in tongue carcinoma cells, as determined with the monoclonal M3/38 monoclonal antibody, was correlated to decreased disease-free survival [80]. Loss of nuclear galectin-3 had been already been observed in colon cancer cells but without observing a prognostic value for this parameter [49]. The mechanisms allowing nuclear localization and exit are the subject of numerous studies that will not be reported here. The alteration of these mechanisms allowing altered cytolocalization of galectin-3 in cancer cells remain to be examined. As stated above, several functions for nuclear galectin-3 have been described, but the implication of nuclear galectin-3 on the cancerous phenotype is still to be explored.

Conclusion

More in-depth studies have been realized and allowed to draw better pictures of expression of galectin-1 and galectin-3 in cancer specimens. The relative importance of stromal galectin-1 and of nuclear or cytoplasmic galectin-3 is emerging and will be the subject of functional studies. These new concepts will help to understand the implication of these two galectins in cancer biology and could constitute the basis for future anticancerous strategies.

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