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90K (Mac-2 BP) and galectins in tumor progression and metastasis

Antonino Grassadonia, Nicola Tinari, Ida Iurisci, Enza Piccolo, Alba Cumashi, Pasquale Innominato, Maurizia D'Egidio, Clara Natoli, Mauro Piantelli and Stefano Iacobelli

Department of Oncology and Neurosciences, Sections of Medical Oncology and Pathology, University "G. D'Annunzio", Chieti 66100, Italy

Galectins and their ligands have been implicated in cell transformation and cancer metastasis, and found to have prognostic value. Mac-2 BP, also known as 90K, is a highly glycosylated, secreted protein extensively studied in human cancer, which binds galectin-1, galectin-3 and galectin-7. High expression levels of 90K are associated with a shorter survival, the occurrence of metastasis or a reduced response to chemotherapy in patients with different types of malignancy. The mechanisms underlying the prognostic significance of 90K and galectins in cancer are far from being understood, although they may be related to the ability of these proteins to interact and, to some extent, modulate cell-cell and cell-matrix adhesion and apoptosis. The resulting scenario is even more complex, as data have been presented that all these proteins might be associated with either a positive or a negative outcome of the patients. It is hypothesised that different galectins and galectin ligands with overlapping or opposite functions, expressed in different tumors during the different steps of the metastatic cascade might play a crucial role in tumor progression.
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Abbreviations: **Mac-2 BP: Mac-2 binding protein; NSCLC: Non-small-cell-lung-cancer; NHL: Non-Hodgkin's lymphomas; HNSCC: Head and neck squamous-cell carcinoma; OS: Overall survival; DFS: Disease-free survival.**

Introduction

Several lines of evidence support a role for galectins in tumor invasion and metastasis [1]. In fact, these proteins have been reported to mediate cell-cell and cell-matrix interactions [2–4], apoptosis [3–7], cell proliferation [8] and angiogenesis [9]. Consistent with a role in cancer progression, data have been presented to show that the expression level of galectins in tumors is associated with the prognosis of the patients.

Many endogenous ligands of galectins have been described, including laminin, fibronectin [4,10], lysosome-associated membrane proteins [11], and Mac-2 binding protein (Mac-2 BP) [12]. Like their complementary galectins, the expression of these ligands has been found to be altered in cancer and associated with the clinical outcome of the patients.

This paper will review the available data on the prognostic value of Mac-2 BP and galectin-1 and -3 in human cancer and discuss the role of these molecules in tumor progression and metastasis. A comprehensive review on galectins and cancer is presented elsewhere in this issue.

To whom correspondence should be addressed: Prof. Stefano Iacobelli, Cattedra di Oncologia Medica, Università "G. D'Annunzio," Pal. Se.Bi., via dei Vestini, 66100 Chieti, Italy. Tel: +39 0871 355 6765; Fax: +39 0871 355 6707; E-mail: iacobell@unich.it

Mac-2 BP/90K

Mac-2 BP refers to a large oligomeric glycoprotein composed of subunits of approximately 90 kDa, identified as a ligand of galectin-3 [12,13]. It has been found to be identical to a previously described tumor-associated antigen released in the culture media of human breast cancer cells and named 90K after its molecular mass [14]. Since this protein binds other members of the galectin family, namely galectin-1 and -7 [15,16], the term of Mac-2 BP appears to be obsolete. Therefore, we propose to use, from now onwards, the name of 90K in order to indicate this multiple galectin binding protein.

90K belongs to the Scavenger Receptor Cysteine-Rich domain (SRCR) superfamily of proteins that includes CD5, CD6, M130, complement factor 1, WC1, and several other proteins implicated in the immune defence and immune regulation [17]. Under non-dissociative conditions and at neutral pH, the average molar mass of the protein is 1000–1500 kDa, since it tends

to self-assemble forming oligomers. These structures appear ring-like shaped in electron microscopy [18]. 90K is synthesized and secreted by different cell types, including hematopoietic cells and glandular or mucosal epithelia [12,13] and is present in the serum and other biologic fluids of normal subjects in the μg/ml range [12,14,19]. Elevated serum levels of 90K have been found in several human diseases, including infection by Hepatitis B virus and Hepatitis C virus, Human Immunodeficiency virus, autoimmune disease and cancer [20–24].

90K in human malignancy

Using a specific and sensitive ELISA [19], it was possible to determine the levels of 90K in the serum of normal subjects and cancer patients [24]. In healthy blood donors, levels of 90K <11 μg/ml are found in 95% of cases. Conversely, serum levels are frequently elevated (>11 μg/ml) in subpopulations of patients with cancer of various origin. In 235 patients with breast cancer, serum levels of 90K correlated with the stage of the disease, with supranormal levels being observed in 26% of patients with stage I disease and in 50% in those with stage IV disease.

Several studies have shown that high 90K levels are associated with a poor prognosis [25–31] (Table 1). In a group of 425 patients with breast cancer in post-surgical follow-up, high 90K serum levels were associated with a shorter overall survival (OS), particularly in patients with positive axillary lymph nodes [27]. At the multivariate analysis, the prognostic value of 90K proved to be independent of that of the other covariates. In another group of 310 patients with advanced, metastatic breast cancer, serum levels of 90K were not correlated with tumor size, tumor histology or estrogen receptor status, but strongly associated with the presence of liver metastasis [27].

The association between 90K and the presence of metastasis has been confirmed in a series of 72 patients with completely resected pathological stage I Non-Small-Cell-Lung-Cancer (NSCLC), in which the expression of 90K has been assessed in the tumor tissue by immunohistochemistry [30]. High expression level of 90K, *i.e.* staining of >50% of the neoplastic cells, was observed in 28% of the cases and found to correlate with a reduced disease-free survival (DFS) and OS. This predictive value of 90K has been confirmed by multivariate analysis. As in breast cancer, the level of protein expression was associated with the incidence of distant metastases. In fact, although 21% of the patients with low 90K expression developed distant metastasis, this percentage raised to 60% for patients with high 90K expression (30).

90K expression has been studied in hematological malignancies as well. In 81 patients affected by Non-Hodgkin's Lymphomas (NHL), circulating serum 90K levels were significantly higher than in healthy blood donors [28]. The Kaplan-Meier analysis of OS showed that patients with low 90K level survived longer than patients with high 90K level. At a multivariate regression analysis, the serum level of 90K was proved to be an independent predictor of survival. In another study, 90K serum levels were determined at the time of initial diagnosis in 116 patients with NHL and 21 with Hodgkin's disease [31]. Serum 90K levels in NHL patients were significantly higher than in healthy blood donors and did not differ from those in patients with Hodgkin's disease. Significantly, high levels were associated to a lack of response to therapy. Similar results were recently reported by Zhang *et al*. [32] who showed a lower response rate to CHOP regimen in NHL patients expressing high

Table 1. Studies reporting the negative prognostic value of high levels of 90K in patients with different types of tumors

Study	*90K serum (S) tissue (T)*	*Tumor*	*Stage*	*N*[1]	*90K correlation with*	*P*
Iacobelli *et al.* (1994)	S	Breast	NED[2]	425	Reduced OS[3]	0.001
			Advanced	310	Liver metastasis	0.009
					Reduced DFS[4]	0.005
Rea *et al.* (1994)	S	NHL[5]	All	81	Reduced OS	0.004
Zeimet *et al.* (1996)	S	Ovary	All	152	Reduced OS	0.03
Fornarini *et al.* (2000)	S	Lymphomas	All	137	Reduced response to therapy	0.011
Marchetti *et al.* (2002)	T	NSCLC[6]	I (NED)	72	Reduced OS	0.0003
					Reduced DFS	0.0001
					Distant metastasis	0.0038
Zhang *et al.* (2003)	S	NHL	All	100	Reduced response to therapy	0.001

[1]Number of patients included in the study.
[2]NED: Non evidence of disease (after surgery).
[3]OS: Overall survival.
[4]DFS: Disease-free survival.
[5]NHL: Non-Hodgkin's lymphoma.
[6]NSCLC: Non-small-cell-lung-cancer.

90K serum levels as compared to those with low 90K serum levels (47.6% vs. 93.6%).

The reported evidences depict 90K as a marker of poor outcome in cancer patients. Is this 90K propensity to negatively influence prognosis in anyway related to its capacity to bind galectins? Important insight to answer this question could come from the studies of the relationships between galectin expression and cancer progression.

Galectins in cancer

The role of galectins (mainly galectin-1 and -3) in cancer has been widely investigated *in vitro* and *in vivo*. Several studies have documented that tumor cells expressing high levels of galectin-3 have an increased metastatic potential [33–38]. In addition, down-regulation of galectin-3 has been found to suppress the tumorigenicity of human breast cancer cells in nude mice [39]. In patients affected by different types of cancer, including those arising from breast, gastrointestinal tract and lung, serum levels of galectin-3 have been found to be significantly higher than in healthy individuals and to correlate with the presence of metastasis [40]. Similarly, increased expression of galectin-1 and/or galectin-3 in the tumor tissue has been found to correlate with the malignant potential of thyroid carcinoma [41,42], glioma [43] and gastrointestinal tumors, including gastric [44], colorectal [45,46], pancreatic [47,48] and hepatocellular adenocarcinoma [49].

90K and galectins in cancer progression

Overall, from these and other data it seems that malignant cell transformation and cancer progression are associated to an increased expression of galectin-1, galectin-3 and 90K. A possible explanation for a mechanistic connection between 90K, galectins and cancer may arise from some recent findings. As originally described by Inohara [50], 90K is able to mediate homotypic cell adhesion and the formation of multicellular aggregates by cross-linking galectin-1 and -3 residues on adjacent tumor cells (Figure 1). This process is critical for cancer cells survival in the bloodstream and is thought to be a key step in metastatic diffusion. Moreover, 90K and galectins have been found to be deposited in extracellular matrix where they might interact with different matrix components, thereby mediating cell adhesion [4,10,11,18,31,51]. Thus, by enhancing the adhesive interactions between tumor cells and extracellular matrix, 90K and galectins may favour the establishment of new tumor colonies. Additionally, increased adhesion to extracellular matrix may help tumor cells to evade apoptosis, a process which has been demonstrated for both 90K and galectin-3. Indeed, when used as a immobilised substrate, 90K caused a significant reduction in chemotherapy-induced apoptosis of Jurkat T lymphoma cells, a finding that has been recalled to explain the lack of response to chemotherapeutic drugs in lymphoma patients displaying high circulating 90K levels [31]. Similarly, data have been presented to show that galectin-3 is able to protect cells against apoptosis induced by a variety of stimuli, including cytotoxic drugs and loss of anchorage (anoikis) [3,4]. As in the case of 90K, galectin-3 can confer a selective survival advantage to metastatic cancer cells.

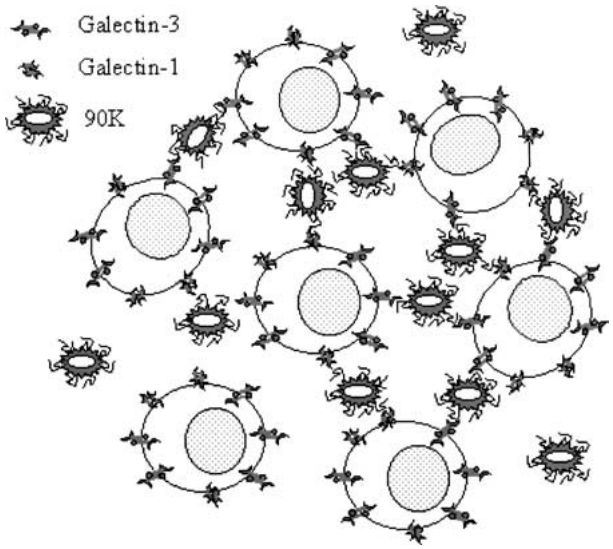

Figure 1. Proposed model of tumor cell aggregation and emboli formation into the circulation mediated by 90K interaction with galectin-3 (Inohara *et al*. 1996) or galectin-1 (Tinari *et al*. 2001). 90K, depicted in its oligomeric ring-shaped structure with carbohidrate chains, acts as a bridge for tumor cells presenting galectin-1 or -3 on the surface (both depicted in their homodimeric form).

The scenario hitherto described, in which 90K and galectins play the role of tumor favouring factors, is complicated by other evidences. In contrast with the described negative influences on prognosis, 90K might possess positive influences as well. For example, cancer cells engineered to overexpress the protein have been found to have a reduced tumorigenic potential in nude mice as compared to their wild-type counterparts [52]. Furthermore, there are several reports of a decreased galectin expression in cancer. For example, up-regulation of galectin-1 parallels down-regulation of galectin-3 during malignant transformation of prostate tissue [53]. Moreover, down-regulation of galectin-3 is observed in breast [54,55], uterine [56] and colon [57,58] adenocarcinomas, and in head and neck squamous-cell carcinoma (HNSCC) [59]. These contradictory observations could be partially explained in the light of the following considerations. In the case of galectins, discrepancies could be dependent on differences in the reagents (antibodies) and procedures (ELISA, Western blot, immunohistochemistry) used for their detection. More importantly, the interpretation of the results obtained for galectin-1 and galectin-3 will only be unequivocal if no additional galectins with overlapping or opposing functions would be expressed in tumors [60]. Finally, the influence exerted by the local expression of potential galectin ligands should be taken into account. In the case of 90K, it should be considered that, with the exception of NSCLC, the prognostic value of this protein has been assessed by the determination of its circulating levels in serum. Actually, it is not known if 90K

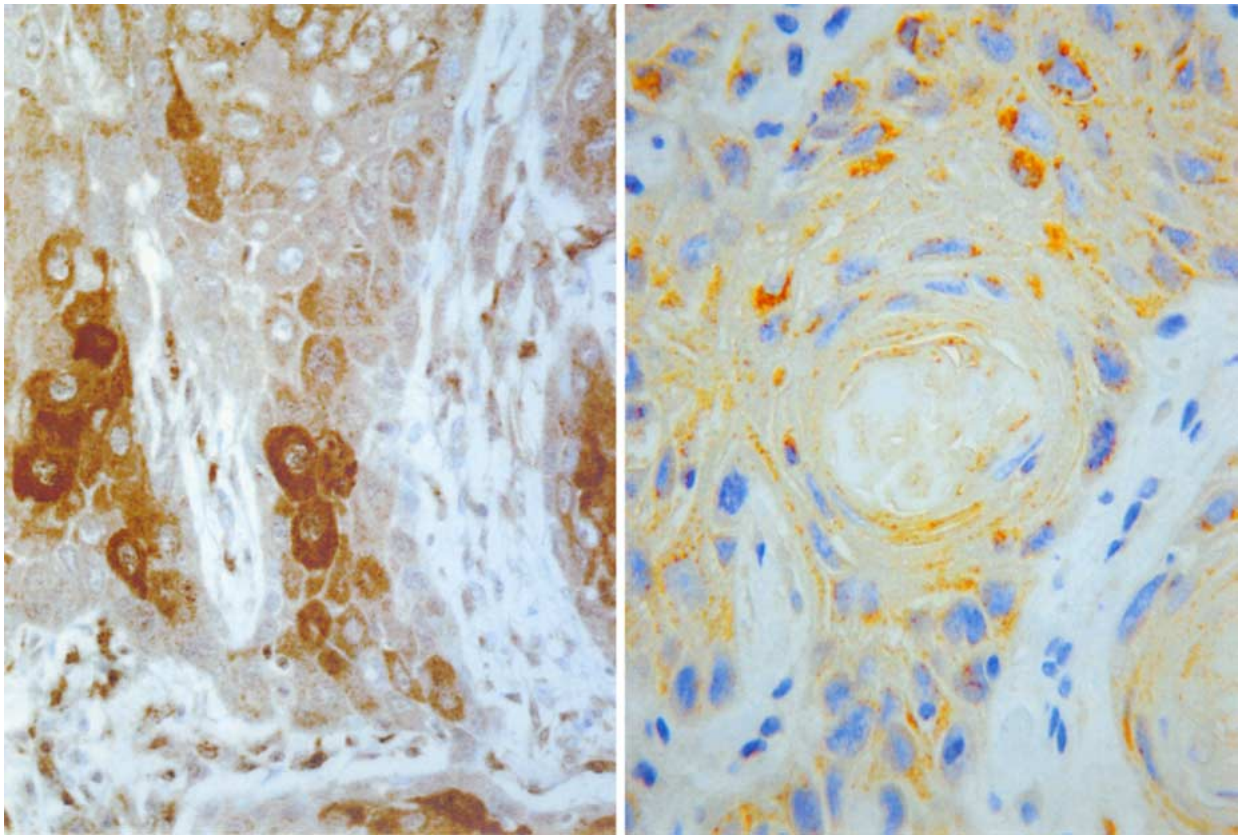

Figure 2. Laryngeal squamous cell carcinoma: positive immunostaining with anti-galectin-3 (left panel) and anti-90K antibodies (right panel). Original magnification: 400×.

measured in the serum does reflect the ability of cancer cells to produce and secrete it into the circulation or if the protein is also produced by host cells in response to tumor progression and if the different origin of the protein may in turn have a differential influence on the course of the disease. Therefore, it seems clear that the role of galectins and the mechanisms underlying their effects during tumor progression are far from being definitely clarified. Similarly, it could be too simplistic to explain the role of 90K in cancer, exclusively as a consequence of its capacity to bind galectins.

A way to escape from this *cul de sac* would be to simultaneously determine the expression of 90K (or other ligands) and galectins in the same tumor, but this kind of analysis is only at the beginning. In a study by Choufani *et al*. [59], a decreased expression of galectin-1, galectin-3 and their binding sites in HNSCC has been found to correlate with an increased tumor aggressiveness. These authors suggested that differences in the expression of these molecules and, probably, in their prognostic significance, may reflect the different embryologic origin of sites from which HNSCC can arise. Consistent with the negative role of the loss of expression of galectin-3 in HNSCC [59] are our recent findings of galectin-3 expression in laryngeal cancer patients [60]. In addition (unpublished observation), in a subgroup of 38 node-negative laryngeal cancer patients, we found that the expression of galectin-3 was positively correlated to that of its endogenous ligand 90K (Figure 2). This is the first evidence of a positive relation between the expression of galectin-3 and that of 90K in the same tumor. Surprisingly, we found that the lack of expression of 90K in the same tumor is associated with the presence of known markers of aggressiveness and a shorter survival.

Conclusions

In conclusion, the take-home message is that there are many galectins and many ligands. In addition, these molecules are differentially expressed in different tissues both normal and neoplastic, and at different stages of tumor progression. It is possible that the expression of galectins and their ligands in different contexts may result in different and even opposite influences on tumor growth and progression. Further studies, less fragmentary and appropriately dimensioned, are needed to clarify the intricate interplay of these molecules, both in physiological conditions and cancer.

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