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EDITORIAL

Innate IgM antibodies to mannose in patients with gastric cancer

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Gastric cancer (GC) remains one of the most common lethal diseases. Every year, about 1 million people fall ill and almost 800 thousand people die worldwide[1]. Surgical treatment has reached its limit. The current advances in immunology and detection of epidermal growth factor molecules (EGFR) in gastric cancer cells allowed to improve the treatment results; however, the metastatic form of this disease is still incurable.

In recent years researchers have focused attention on mannose (D-Man), a hexose which is an essential component of all glycoprotein N-chains involved in normal physiologic and pathologic processes. Man is recognized by mannose-binding lectin (MBL) and the mannose receptor (MR), which is expressed in dendritic cells and macrophages.

The role of mannose in gastric cancer progression

An article was recently published in *Nature*[2] on the effective use of free Man in suppressing malignant cell growth *in vivo* and *in vitro*. The authors suggested that Man is transported into a cell *via* the same mechanisms as glucose, but Man disrupts glucose metabolism inside the cell, which reduces the expression of the Bcl-2 family proteins, which in turn kills tumor cells. There was no such effect when adding other monosaccharides, such as galactose, fructose, fucose, and glucose.

In a corollary study, Gu et al.[3] conducted an in-depth study of the free (not included in complex glycans) D-Man content in the sera of 159 patients with esophageal adenocarcinoma using liquid chromatography-mass spectrometry (LC-MS/MS). Patients with high D-Man levels in the serum had a reduced risk of death compared to patients with low levels of D-Man [relative risk (RR) = 0.44, 95% confidence interval (CI) = 0.25–0.77; $P < 0.01$]. Accordingly, the 5-year survival was 66.4% and 44.6% in the two groups, respectively. The overall median survival was significantly longer in the high-D-Man group compared to the low-D-Man group (> 123.4 months *vs.* 36.9 months; $P = 0.01$). A similar trend in recurrence-free survival was observed. Specifically, the risk of recurrence was two times lower in the sera of patients with high D-Man levels compared to low D-Man levels (RR = 0.51, 95% CI = 0.29–0.91; $P = 0.02$). The 5-year recurrence-free survival was in the high- and low-D-Man groups was 69.8% and 46.6%, respectively. The recurrence-free median survival was > 123.4 months versus 32.9 months, respectively ($P < 0.01$). In addition, an elevated D-Man content was associated with better survival among patients with early (RR = 0.40, 95% CI = 0.16–0.98; $P = 0.05$) and late stage esophageal adenocarcinoma (RR = 0.45, 95% CI = 0.21–0.92; $P = 0.03$). Moreover, patients with a BMI < 30 kg/m^2 had better survival (RR = 0.46, 95% CI = 0.22–0.93; $P = 0.03$); there was no survival benefit in patients with a BMI > 30 kg/m^2.

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Several studies determined the effect of D-Man on tumor cells *in vitro.* Sha et al.[4] incubated lung adenocarcinoma cells with Man, carboplatin, and Man + carboplatin. Sha et al.[4] reported that Man significantly inhibits A549 cell proliferation, potentiates the anti-tumor effect of carboplatin, and enhances tumor cell apoptosis.

Fan et al.[5] combined the use of chemotherapy drugs and D-Man [methotrexate-Man nanoparticles (MTX–Man NPs)]. It is presumed that such a particle should be easily recognized by a tumor cell *in vitro* and *in vivo*. MTX–Man NPs had better accumulation in tumor tissues and had a mutually reinforcing effect[5].

Macrophage MR

The MR is located on the surface of dendritic cells and macrophages. MR recognizes terminal Man residues of different glycoconjugates and has an important role in innate and adaptive immunity[6].

Liu et al.[7] studied MR expression in gastric tumors and adjacent normal mucosa in 120 patients with gastric cancer (GC) using an immunohistochemical method. Liu et al.[7] demonstrated a strong reaction in the tumor cell nuclei and cytoplasm and a weak or negative reaction in the surrounding gastric mucosa. An increase in MR expression was directly correlated with tumor size, stage, and the presence of regional metastases. There was no association with gender, age, localization, Lauren's classification, distant metastases, or lymphovascular invasion. These results have been confirmed by determining the microRNA level, which is responsible for MR expression in GC tissues and in the surrounding mucosa. An analysis of recurrence-free and overall survival indicated that patients with pronounced expression of MR in the tumor had significantly worse results when compared to patients with weak expression ($P = 0.001$ and $P = 0.003$, respectively). Liu et al.[7] emphasized that the significance of non-zero MR expression in normal mucosa is unknown, but may reflect pre-cancerous changes.

Liu et al.[8] studied the expression of D-Man-containing glycans using *Narcissus pseudonarcissus* lectin (NPL) and macrophage MR using anti-CD206 antibody in GC cells and tumor macrophages on paraffin blocks using immunohistochemical analysis. Unaffected gastric mucosa was designated as the control. The number of NPL+ and MR+ macrophages was significantly higher in the tumor tissues than the normal adjacent mucosa. The D-Man-containing glycan expression also significantly prevailed in the tumor tissues. They found a weak negative correlation between the expression of both molecules in the tumor macrophages ($r = -0.363$, $P = 0.009$). An increased number of NPL(+) macrophages in the tumor tissues was a significant favorable factor in long-term survival, whereas an increased number of MR(+) macrophages was an unfavorable factor. It is interesting to note that the penetration of NPL(+)-macrophages into the tumor tissues was better compared to MR(+) macrophages. Based on the multi-factor analysis, the affected lymph nodes ($P = 0.004$) and excess MR(+) macrophages ($P = 0.033$) in the tumor tissues were negative predictors of overall survival. At the same time, the expression of D-Man-containing glycans in GC cells did not affect overall survival.

Ding et al.[9] measured the concentrations of soluble Man receptor (sMR) and soluble scavenger receptor (sCD163) in the sera of 143 patients with GC, 66 patients with benign stomach diseases, and 59 healthy donors. The expression of sMR and sCD163 was significantly higher in patients with GC compared to the control group ($P < 0.0001$). Elevated preoperative levels of sMR and sCD163 were highly correlated with a worse prognosis, the presence of regional and distant metastases, and the levels of tumor markers (CEA, CA19-9, CA72-4, and CA-125). Ding et al.[9] emphasized that the basis for these results was not clear, but might reflect receptor desquamation from active macrophage surfaces and entry into the bloodstream. Ding et al.[10] reported similar increased sMR and sCD163 results when studying 163 patients with colorectal cancer, in which the markers were measured not only in blood, but also in tumor paraffin sections using an immunohistochemical analysis. The authors hypothesized that increased MR expression in the tumor tissues reflected stimulation of M2 macrophages, which are responsible for enhanced tumor growth.

MBL

Baccarelli et al.[11] were among the first to report on MBL genetic polymorphisms in patients with GC. Baccarelli et al.[11] provided data comparing 305 GC cases with 427 controls in the Polish population. MBL deficiency was studied in six different haplotypes by identifying alleles in the MBL2 gene promoter. The HYD haplotype was associated with an increased risk of GC compared to the most common HYA haplotype (RR = 1.9, 95% CI = 1.1–3.2; $P = 0.021$). Based on diplotype analysis, YA/D carriers had the highest risk of developing GC compared to the YA/YA diplotype [odds ratio (OR) = 3.0,

95% CI = 1.2–7.1; P = 0.015]. Further analysis of the synergy between the effects of the MBL2 defect and interleukin (IL)-1B revealed a 3.5-fold risk (OR = 3.5, 95% CI = 1.6–7.6; P = 0.001) with a combination of the HYD MBL2 haplotype and IL-1B genotype. At the same time, Wang et al.[12] did not detect a significant difference among the entire cohort of patients and donors in a study involving the MBL2 gene polymorphism in 388 patients with GC and 144 healthy donors in the Japanese population. Moreover, Xie et al.[13] reported no association between the MBL gene polymorphism and GC in a meta-analysis of 43 published studies.

Therefore, free D-Man competes for incorporation into glycolytic processes in tumor cells, which ultimately decreases the efficiency of metabolism. Alternatively, the positive effect is explained by increasing the content of fucosylated glycans biochemically synthesized *via* GDP-D-Man, an important modulator of the gut microbiome and immune system with potential anti-inflammatory activity[14]. An increase in the D-Man-containing structures expression on the membrane surface of tumors and other cells facilitate recognition of the MR and MBL. The latter, in turn, triggers an alternative complement activation pathway to recognize tumor cells. Of note, disease development is dependent on the MBL gene polymorphism, which likely affects the genetic predisposition to this type of cancer, although this was not confirmed by the meta-analysis[13].

Anti-glycan antibodies

MR and MBL are not the only proteins that recognize Man. Indeed, there are natural antibodies that are components of innate immunity.

Innate IgM class antibodies produced by B1 cells are a specific category of antibodies that are characterized by polyreactivity and the lack of V(D)J recombination compared to antibodies produced by B2-lymphocytes. Moreover, synthesis of innate antibodies cannot be stimulated by immunization. Many of these antibodies target glycans[15], including tumor-associated Tn, TF, and SiaTn, which are overexpressed in gastrointestinal tract tumors[16] - so called aberrant glycosylation, which usually consists of carbohydrate chain shortening on tumor cell membranes[17]. In a healthy individual, the aberrant oligosaccharides are recognized by innate antibodies, initiating the elimination of constantly emerging transformed cells (i.e., a supervision function). At the same time, in patients with cancer, including cancers of the gastrointestinal tract, the level of antibodies to aberrant glycans is often reduced[18].

Using a printed glycan array[19] in patients with GC, we demonstrated a deficiency in anti-glycan antibodies to Manβ and Manβ1-4GlcNAcβ, which are core fragments of all glycoprotein N-chains. The deficiency increased with age (**Table 1** and **Figure 1**). The low level of anti-Man IgM in patients with GC confirms our earlier report[20] and is consistent with other data pertaining to innate immunity deficiency associated with D-Man (MR, and in some cases MBL as a part of innate immunity).

We suggest that patients with GC have a deficiency in natural immunity that leads to a violation of tumor cell elimination that can be corrected with the help of specific innate immunoglobulins (**Figure 2**).

Table 1 Median relative fluorescent units (RFUs) of M class antibodies to Manβ and Manβ1-4GlcNAcβ in serum samples from patients and healthy donors. Bold indicates a P < 0.05

Glycan	Median RFU (MAD)		P
	Patients (n = 235)	Donors (n = 76)	
Manβ	3,018 (257)	5,590 (443)	**0.0001**
Manβ1-4GlcNAcβ	3,940 (291)	6,375 (445)	**0.0075**
Group ≤ 45	Patients (n =22)	Donors (n = 68)	
Manβ	3,563 (339)	6,818 (497)	**0.0331**
Manβ1-4GlcNAcβ	7,382 (444)	6,791 (490)	0.4851
Group ≥ 45	Patients (n = 213)	Donors (n = 8)	
Manβ	688 (71)	2,819 (245)	**0.0026**
Manβ1-4GlcNAcβ	1,366 (176)	3,796 (283)	**0.0005**

MAD, median absolute deviation.

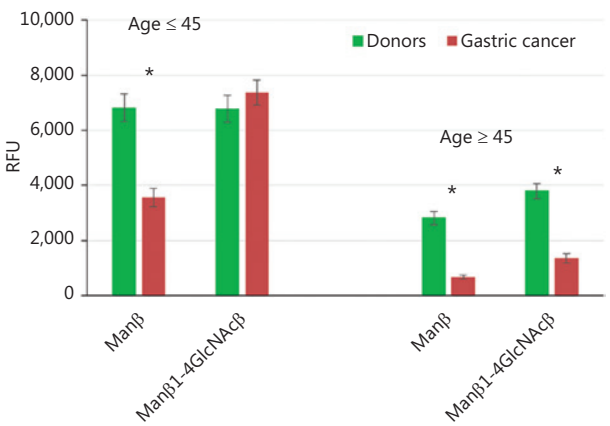

Figure 1 Antibody levels to Manβ-containing glycan in groups of donors and patients with gastric cancer ≤ and ≥ 45 years of age. *P < 0.05.

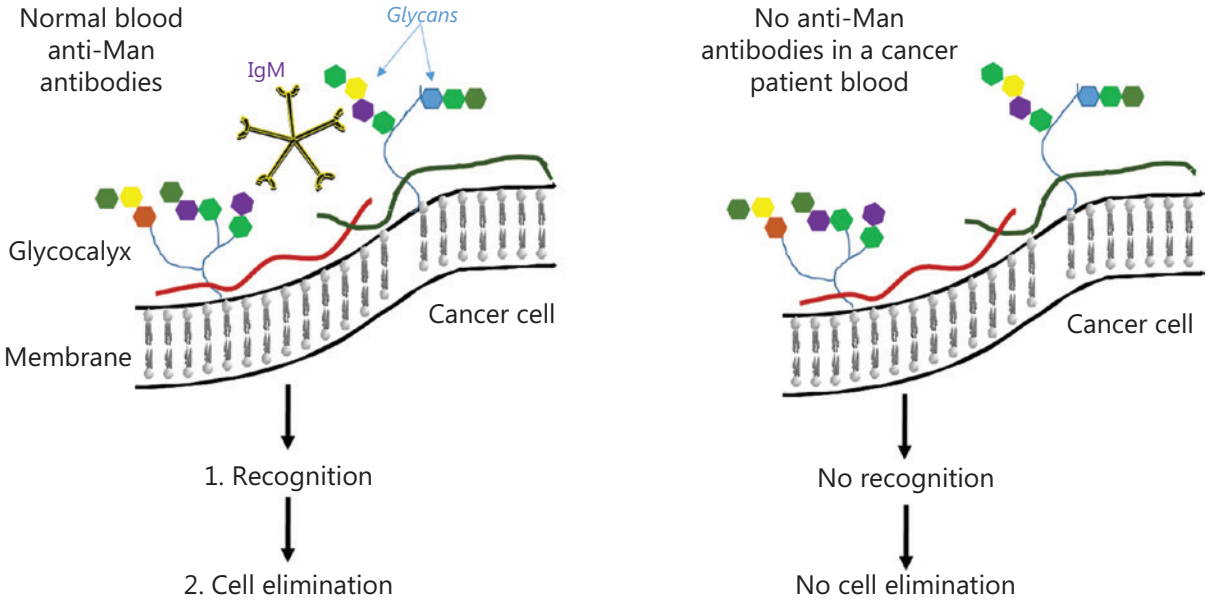

Figure 2 Specific IgM binds to tumor-associated mannose-containing glycans followed by eliminating cancer cell.

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Conflict of interest statement

No potential conflicts of interest are disclosed.

Author contributions

Nikulin, M.P. and Shilova, N.V. – designed the study, manuscript preparation.
Lipatnikov, A.D. and Semyanikhina, A.V. – performed experiments and statistics.
Stilidi, I.S., Bovin, N.V. and Tupitsyn, N.N. – conceptualization, manuscript preparation.

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