

The Various Aspects of Sialylation and α -1,3-Galactosylation in Human Body

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High sialylation level can be “magic formula” how to slow down ageing process, but *N*-glycolylneuraminic acid, one type of sialic acid, can shorten the life. *N*-glycolylneuraminic acid was eliminated in human evolution, resulting in the production of antibodies to this sugar. Similarly, α -gal epitope, the galactose bound by an α -1,3-linkage to LacNAc, was eliminated in human evolution, resulting in the production of antibodies. This commentary highlights medical and biotechnological advantage of these two evolutionary events and discuss consequences connected with cancer, age and nutrition.

It is generally accepted that sugar moieties, *N*-terminally modifying proteins, are involved in determining the protein structure, function, activity and immunogenicity, and that, cell membrane glycosylation pattern is characteristic for different cell types, different tissue and different species. In the case of surface oligosaccharide sequences, it is possible to find exceptional intraspecies variations (e.g., ABO blood groups within humans and other primates), but interspecies variations are much more extensive, and sometime, a specific type of terminal glycan can be permanently eliminated from an entire evolutionary clade. The human-specific loss of *N*-glycolylneuraminic acid, that is common in mammals, and the loss of the terminal α -1,3-galactose in Old World primates are two of these extraordinary cases. The loss of *N*-glycolylneuraminic acid (Neu5Gc) occurred 2-3 mya, just before the emergence of the genus *Homo*, by deletion in gene encoding enzyme cytidine monophosphate *N*-acetylneuraminic acid hydroxylase (CMAH) which is located on human chromosome 6 [1]. Nonfunctional CMAH is responsible for the fact that human glycan moieties wear on the end *N*-acetylneuraminic acid (Neu5Ac) instead of Neu5Gc, and that human immune system is able to produce antibodies against glycostructures capped by Neu5Gc [1]. The loss of the terminal α -1,3-galactose (α -Gal epitope) occurred before the divergence of apes and monkeys, 28 mya [2]. The α -1,3-galactosyltransferase (α 1,3GalT), the mammalian enzyme responsible for the synthesis of α -Gal epitope, has two homologues in human genome—GGTA1P pseudogene in chromosome 9; and GGTA2P pseudogene in chromosome 12. Additionally, isogloboside 3 synthase (chromosome 1), the enzyme responsible for the synthesis of a glycolipid containing terminal α -1,3-galactose, is also inactivated. The reason for the inactivation of these three genes is not clear, but had to be strong because apes and humans still produce large amount of anti-Gal antibodies (1% of circulating human IgG), and these antibodies appear within first 6 months of life [3]. Human body produces permanent antibodies against Neu5Gc and α -Gal oligosaccharides exposed on cell and protein surfaces. In light of this, artificially-induced Neu5Gc-sialylation and α -1,3-galactosylation could represent promising tools for cancer immunotherapy. Immunotherapy is presupposed as ideal solution in anticancer research, because there is belief that antibodies against premalignant or malignant tissues can set up cancer elimination [4]. However until now, the research on this field gives no convincing evidence that this approach is generally effective. It seems that moderate anti-tumor immune reaction is optimal for tumor growth, rather than being inhibited, and that many *de novo* tumors are probably dependent, at least early in their progression, upon eliciting an immune reaction. Contrary, very high natural levels of these antibodies might actually

inhibit tumor growth and be protective in humans (Figure 1) [5]. In light of this, α -1,3-galactosylation has better position (1% of circulating human IgG) and several authors have already reported the increased efficiency of melanoma tumor vaccines genetically engineered to express the α -gal epitope [6-8]. Anti-Neu5Gc antibodies are in much lower level, from this reason, the incorporation of Neu5Gc to cancer cells is responsible for the antibody-mediated inflammation that is insufficient to kill the tumor, but contrary it stimulates angiogenesis and provides growth factors for cancer tissue [5]. Additionally, a typical feature of malignant human tumors is the increased expression of sialylated oligosaccharides, in this case, Neu5Gc is quite “lost” among Neu5Ac acids, and binding of Neu5Gc-antibodies is less effective in “Neu5Ac noise”. As the results, quite high level of Neu5Gc-antibodies is needed to suppress tumor growth [5]. High level of sialylation is not only cancer marker, it has also positive impact on ageing. Negative charge

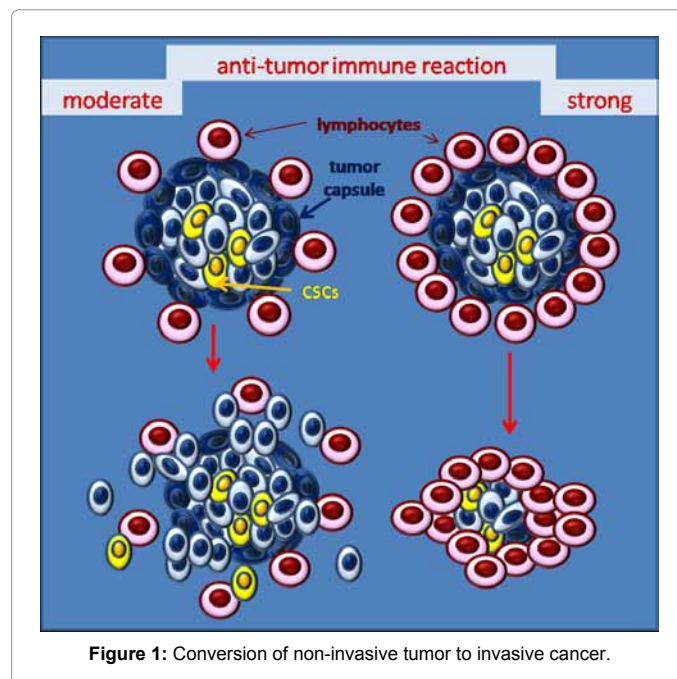


Figure 1: Conversion of non-invasive tumor to invasive cancer.

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Received August 02, 2012; **Accepted** August 04, 2012; **Published** August 08, 2012

Citation: Nahalka J, Talafová K (2012) The Various Aspects of Sialylation and α -1,3-Galactosylation in Human Body. J Glycomics Lipidomics 2:e108. doi:10.4172/2153-0637.1000e108

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of the sialic acids protects from cell-toxic hydrophobic aggregation of cytosolic proteins, during intensive growth stage (for example, during the growth of metastatic cells). Danger hydrophobic aggregations are probably the reason for fact that the level of sialic capping is major determinant of circulating half-life of serum glycoproteins and cells. Desialylated glycoproteins, with free galactose residues, are recognised by the liver asialoglycoprotein receptors, which remove bound asialoglycoproteins from the serum by endocytosis [9]. In the process of human red blood cell ageing, cryptic α -gal epitopes are exposed de novo on the cell surface by the action of macrophage hydrolases [10]. For this reason, a few hundred α -gal epitopes are present on the surface of normal senescent red blood cells. Naturally occurring anti-Gal antibodies bind this epitope, enhancing the phagocytosis of red blood cells [10]. In other words, sialylation is native defense against hydrophobic physiological aggregation and exposed galactose, especially α -gal, is marker for losing of the negative surface charge. It is curiosity that high sialylation capping, induced during intensive metabolism of metastatic cells, protecting against hydrophobic interaction, tells to immune system: "I am young, healthy and self cell". Human nutrition has to be reason that is possible to find some amount of Neu5Gc on human tissues. The cancer cells, with intensive metabolism and higher sialylation, concentrate Neu5Gc. Neu5Gc-antibodies bind to the surface, eliciting weak immune reaction, and the antibody-mediated inflammatory makes the tumor progression by released growth factors [5] and by lymphocyte-mediated shuttling that allows lymphocytes to physically "drag" tumor cells to different sites [11]. The major source of Neu5Gc seems to be red meat and less source the milk products [1]. The red meat and the milk products are also source of the α -Gal epitope, and their very often consumption could lead to α -Gal epitope incorporation to glycans (especially by transglycosidases), and to weak immune reaction and similar tumor progression. The scenario of insufficient immune response to kill the tumor is unwanted, however it was already demonstrated that strong immune reaction can be medically and biotechnologically used. Intratumoral injections of α -gal glycolipids led to in situ conversion of melanoma lesions into effective autologous vaccine [12].

The red meat and the milk products are sources of oligosaccharides that mediate moderate immune reaction and tumor progression. Contrary, strong immune reaction achieved by glyco-engineering of cancer cell surfaces can be medically used to kill the tumors. CSCs means cancer stem cells (yellow cells), lymphocytes (in red color) physically "drag" them to different sites (Figure 1).

In light of above mentioned philosophy, the initiation of sufficient immune response, by glyco-engineering of cancer cell surfaces, has chance to provide tool for directing the fate of cancer cells to decomposition and death. It opens space for biotechnology that can provide α -galactosylation and Neu5Gc-sialylation by enzymatic or genetic approaches. Enzymatic approaches need tools for high-throughput screening of the enzymes. In article of this issue, our laboratory presents high-throughput methodology that uses bacterial sialic acid binding adhesin from *Helicobacter pylori* (Sab A) for in situ monitoring of sialylation level [13].

The author appreciate the support from the project: Centre of excellence for white-green biotechnology, ITMS 26220120054, supported by the Research & Development Operational Programme funded by the ERDF.

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