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REVIEW ARTICLE

Is α-N-acetylgalactosaminidase the key to curing cancer? A mini-review and hypothesis

Ehsan Saburi[1,2], Jalil Tavakol-Afshari[3], Sajjad Biglari[1], Yousef Mortazavi[4,5]

[1]Molecular Medicine & Genetics Department, Faculty of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran; [2]Department of Cancer Research, Nastaran Center for Cancer Prevention, Mashhad, Iran; [3]Immunogenetics Department, Bu-Ali Research Institute, Mashhad University of Medical Sciences, Mashhad, Iran; [4]Medical Biotechnology & Nanotechnology Department, Faculty of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran; [5]Cancer Gene Therapy Research Center, Zanjan University of Medical Sciences, Zanjan, Iran

Summary

In the constant battle against cancer cells, macrophages are of great importance. Their activation is achieved through various mechanisms such as Vitamin D binding protein (VDBP or Gc). After undergoing modifications via enzymes secreted by stimulated lymphocytes, VDBP is modified into Macrophages Activator Form/Factor (Gc-MAF). Some studies (particularly those focusing on cancer) have reported that an enzyme known as α-N-acetylgalactosaminidase (nagalase) facilitates the deglycosylation of Gc-MAF, which in turn inhibits the activation of macrophages. The aim of this review was to evaluate studies associated with nagalase and its escalation in various diseases and to propose hypothetical solutions in order to neutralize the effects of nagalase in cancer patients.

Key words: *α-N-acetylgalactosaminidase, Macrophages Activator Factor, neoplasms, Vitamin D Binding Protein*

Introduction and background

Macrophages play a key role in the fight against cancer cells but only following a complex activation mechanism. After being modified by sialidase and galactosidase, respectively secreted by T and B lymphocytes, Vitamin D binding protein (also known as Gc Globulin, Gc Protein and D Binding Protein) acquires the ability to activate macrophages; this form is referred to as Gc-MAF (Group specific component macrophage activating factor) [1,2]. This 56kD member of the albumin super-family is secreted by the liver and promotes the superoxide generating capacity of macrophages [2], increases general and antibody-dependent phagocytic ability and also induces translocation of FcgR1 and FcgR2 receptors [3]. Gc-MAF's anti-tumor effects include anti-angiogenic properties [4], adjuvant effects, osteoclast activation, facilitation of chemotaxis (with the help of C5 derived peptide) and the scavenging of circulating G-actin [5]. Vitamin D binding protein has been proven to affect host susceptibility to many diseases, including but not limited to chronic obstructive pulmonary disease (COPD) [6], endometriosis [7], osteoporosis [8], autism [9], systemic lupus erythematosus (SLE) [9] and multiple types of cancer such as melanoma [10], squamous cell carcinoma [11] and oral cancer [12]. In most cases GcMAF degradation was mediated by an enzyme called α-N-acetylgalactosaminidase (nagalase).

Correspondence to: Yousef Mortazavi, PhD. Medical Biotechnology & Nanotechnology Department, Faculty of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran.
Tel: +989123892427, Fax: +982122542939, E-mail: ymortaza@zums.ac.ir & youmort@yahoo.com
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Nagalase: Structure & Function

Increased serum levels of nagalase have been reported in many cancer patients, therefore it has been suggested that this enzyme is responsible for the deglycosylation of Gc-MAF [13] (Figure 1). Even though the intracellular (lysosomal) form of nagalase is vital for proper hepatic cell function, the extracellular form (secreted by cancer cells) seems to only benefit the progression of cancer [14]. The lysosomal form acquires optimal activity in acidic environment (pH~5) but the extracellular form functions in plasma pH levels, approximately 7.4 [8]. It has not been clarified if the structure of circulating nagalase is the same as the natural lysosomal form. It may be that differences in its active site, carboxylic or aminic-ends, its glycosylated side branches or the addition of a cofactor/chaperone causes this newfound ability to function in plasma pH levels [14]. Also needing clarification is the mechanism of how nagalase is secreted. Cancer cells may possess the ability to modify the structure of the enzyme to facilitate its secretion. Profound evidence of the role of O-Linked connections between adjacent cancer cells or between cancer cells and the extracellular matrix (ECM) exist. Based on the role of nagalase in glycoprotein destruction, as well as the type of cell-cell/cell-ECM connections, nagalase can be considered as an important biomarker in cancer.

Nagalase as a cancer biomarker/treatment prognosis marker

Previous studies have shown increased plasma levels of nagalase in many types of cancer (Table 1). Yamamoto and colleagues reported this increase for the first time in various types of cancer patients in 1996 [15], and this increase was later confirmed in multiple studies by other authors [11,16]. Further studies showed a correlation between increased nagalase and the pathogenesis of cancer cells in cell cultures and animal models [14]. Plasma nagalase levels are affected by many different factors including the cancer type and its severity, metastatic ability, if the tumor is primary or recurrent, and also the chosen cancer therapy regimen [12,16]. In most cases, cancer therapy causes reduced plasma nagalase levels in a variety of cancers. In a study performed by Thyer and colleagues, the plasma nagalase levels were almost halved. Today the strategy of many promising cancer therapies are to increase Gc-MAF levels and in doing so, hoping to reduce plasma nagalase. Although administration of intravenous Gc-MAF is a yet unapproved therapy, due to prior evidence of its success it is performed by some practitioners around the world [17].

According to some articles and research centers, the recommended reference range of nagalase in the serum of healthy people is between 0.32 and 0.95 nM/min/mg of substrate, although in some articles the normal range is slightly lower (up to 0.65nM/min/mg). Certainly more research is required to ascertain a universal and reliable normal threshold.

Nagalase in cancer cell lines

A few studies have investigated the level of nagalase in cancer cell lines and their results indicate that values differ in various cell lines. Despite normal levels in fibroblasts and keratinocytes such as the human embryonic lung fibroblasts (HEL299), human gingival fibroblasts (HGF) and human gingival keratinocytes (HGK), elevated levels of nagalase were seen in human salivary gland adenocarcinoma cell lines (HSG) and squamous cell carcinoma cell lines (SCCTM) [18,19].

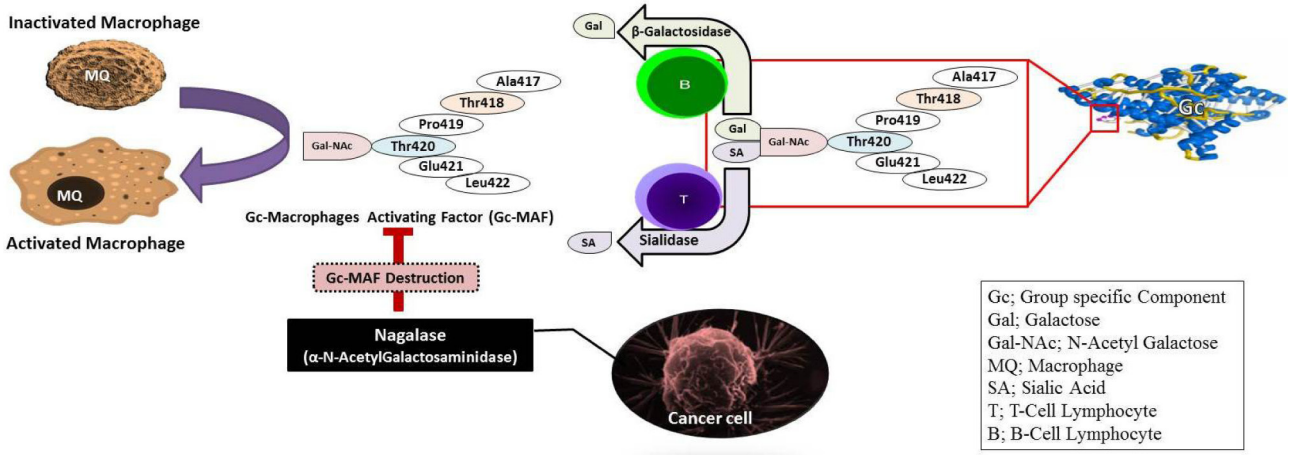

Figure 1. The cascade forming Gc-MAF from Gc protein and its destruction by Nagalase.

Table 1. Studies showing serum nagalase levels in patients with various diseases

References	*Year*	*Disease (number of patients)*	*Nagalase concentration in serum (nM/min/mg)*	
			Pre-treat. (±SD)	Post-treat. (days/or NG)
Yamamoto et al [48]	1995	HIV-infected patients (14)	3.47 (±3.8)	0.24 (NG)
Yamamoto et al [15]	1996	Various types of cancer (20)	1.76 (±1.1)	0.23 (NG)
Koga et al [49]	1996	Malignant tumors (11)	3.35 (NM)	0.24 (NG)
Yamamoto et al [13]	1997	Systemic Lupus Erythematous-SLE (33)	1.78 (±0.97)	0.29±0.10 (NG)
Yamamoto et al [12]	1997	Oral cancer with squamous cell carcinoma (36)	3.03 (±2.01)	0.29±0.1 (NG)
Reddi et al [11]	2000	Uterine cervical cancer as squamous cell carcinoma-SCC (210)	2.67 (NM)	1.13 (NM)
Nakagawa et al [50]	2003	HIV-infected patients (12)	NM	Decrease
Yamamoto [20]	2005	Acute influenza in the third day (4)	1.73 (±0.17)	0.50±0.05 (60)
Yamamoto [21]	2006	HIV-infected patients (24)	5.37 (±2.91)	0.23 (NG)
Yamamoto et al [51]	2008	Non-anemic prostate cancer (16)	3.57 (±0.93)	<0.68 (175)
Greco et al [10]	2009	Melanoma in stage III (35)	> 8 (NM)	<4 (NM)
Bradstreet et al [9]	2012	Autism spectrum disorders-ASD (40)	1.93 (±1.21)	1.03±0.67 (~100)
Thyer et al [17]	2013	Advanced neoplasms (20)	2.84 (±0.26)	1.59±0.17 (263±45)
Gulisano et al [52]	2013	Various types of cancer (20)	2.84 (±0.26)	1.59±0.17 (7)

NG: normal group, NM: not mentioned

Nagalase in microorganisms

There is abundant evidence in favor of the presence and role of the nagalase in different pathogens. The pathogenesis of some viruses such as *Influenza* [20], *HIV* [21] or *Herpes Simplex Virus-HSV* [22] is attributed to nagalase. There is evidence of this enzyme playing a crucial role in various bacteria, especially in Intestinal Microbiota such as *Bifidobacterium spp.* (particulary *B. longum*) [23], *Streptococcus mitis* [24] or *S. pneumonia* [25], *Enterococcus faecalis* [26], *enterobacteria spp.* [27], *Pseudomonas aeruginosa* [28], *Paenibacillus spp.* [29], and emphatically *Clostridium perfringens* [30] and others. The use of nagalase for ECM destruction has been proven in trophozoites of *Entamoeba histolytica* [32], although nagalase has been seen in other parasites too, such as *Toxoplasma gondii* [33], *Giardia lamblia* [34], *Schistosoma mansoni* [35], *Clonorchis sinensis* [36] and even in a type of mosquito, *Phlebotomus papatasi* [37]. Although research about the presence of nagalase in fungi is limited, some studies show its presence in *Aspergillus spp.* (*especially A. Niger*) [38], *Penicillium oxalicum* [39], *Streptomyces spp.* [40] and *Acremonium spp.* [41]. Some studies have discovered an analog or similar enzyme to nagalase beta-N-acetylgalactosaminidase in *Bacillus spp.* [42] Based on the abundant evidence indicating the presence of nagalase in different pathogenic organisms, additional studies need to clarify its exact mechanism of action.

Hypothesis

Reduced expression of nagalase

Gene silencing is one of the many categories of gene therapy and has proven to be a field of increased importance in previous decades. However the occurrence of unexpected side effects has caused it to not yet be a globally approved treatment. Gene silencing can be achieved through siRNA or shRNA or crisper-cas9, but these methods are still limited to research laboratories and have not yet found their place in clinical therapy [43,44]. We predict that with the use of gene silencing methods, reduced expression of nagalase and consequently reduced cancer cell invasion capability can be achieved. The most probable option using this method would be with the help of siRNA/shRNA targeted for the NAGA gene, its enhancer or a hypothetical sequence that enables nagalase to be secreted.

Enzyme inhibition

In the case that a new pseudo-substrate (a glycoprotein with an α-N-acetylgalactosamine branch) could be presented to nagalase as a competitive/alternative inhibitor, the decoy could reduce the deglycolysation of Gc-MAF. The same idea lies behind the current IV administration Gc-MAF, albeit with greater financial cost. Maybe a non-competitive or uncompetitive enzyme inhibitor could be used to reduce nagalase activity as

Ayers and colleagues have suggested [45]. Also, changes in the post-translation phase may be effective such as chaperone manipulation [46].

Therapeutic and metastasis tracing monoclonal antibodies

Synthesis of a monoclonal antibody against plasma nagalase could offer major benefits, including the ability to increase the susceptibility of nagalase to be identified, presented and eliminated by the patient's immune system. In the case that these antibodies are labeled, distant and local metastasis could be discovered (previous studies have shown the highest levels of nagalase can be located around cancer cells), therefore likely benefits could include faster and more effective detection and treatment of metastatic sites. On the other hand, based on this and the fact that nagalase prefers an acidic environment, if pH could be locally increased around such sites, loss of enzyme function could be achieved. This local inhibition would be especially effective in synergy with IV administration of Gc-MAF [47] or in conjunction with targeted chemotherapy (nagalase antibody attached liposomes transporting chemotherapy drugs).

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Conflict of interests

The authors declare no conflict of interests.

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