# Antimetastatic therapy targeting aberrant sialylation profiles in cancer cells

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## Abstract

Neoplasm metastases involve a fixed cascade of pathological processes, and are responsible for more than 60% cancer deaths worldwide and can only be controlled or inhibited by drugs now. Antimetastatic drugs targeting aberrantly sialylated in tumors have involved about a quarter of a century and might be a future therapeutic option apart from currently utilized antimetastatic drugs, such as antivascular and matrix metalloproteinase (MMP) inhibitors. Since neoplasm tissues often manifest high levels of sialic acids and sialyl antigens or glycoligands, and some types of sialic acid analogue, such as N-glycolylneuraminic acid (Nau5Gc) occurred in most tumor tissues, is absent in common humans, more attentions are needed to work with new therapeutic approaches to target these changes. Previously preliminary data have shown some compounds that inhibit some pathways of sialic acids can inhibit the tumor metastasis in vitro and tumor metastasis in experimental animal models. This type of pharmacological work can be helped by glycome investigations in order to deep understanding their mechanisms. As the *central dogma* of glycobiology is still unknown, some fundamental questions related to carbohydrate itself are even more welcoming and decisive to our understanding to nature of cancer. These types of work also need mathematical analysis of data. In this review, we will document and discuss the latest experimental therapeutic data and their clinical significance between cancer pathological profiles and therapeutics benefits.

## Introduction

Cancer is one of the high-mortality diseases, which causes the annual deaths listing among the top 5 mortality in almost all countries. Unlike cardiovascular diseases, the treatment beneficiary for cancers especially for epithelial carcinoma has been improving slightly over the past several decades.[1-3] Neoplasm metastasis is one of the fatalist characters responsible for these unsatisfactory of therapies and more than 60% cancer deaths and can be hopefully only controlled by drugs. Paradoxically to our efforts and expectations, except some antibodies, no obvious improvements and therapeutic benefits by conventional antimetastatic drugs [usually antivascular agents or matrix metalloproteinases (MMPs) inhibitors] have been achieved until now. Therapeutic benefits in late-staged or aged cancer patients are especially poor and useless.[1-3] Clinical anticancer drug therapies currently in use have been mainly focusing on primary tumor growth rather than specifically targeting pathologic courses of metastases relevantly.[4] Finding important drugs targeting specifically to neoplasm metastases is essential and indispensable.[5] It nevertheless needs changing our focus from targeting vascularity and MMPs into more metastatic-relating molecules.

According to general points of view, good antimetastatic therapy must be based on thorough understanding of metastatic biology and pathology. Antimetastatic drugs extensively studied nowadays have been mainly focusing on angiogenesis and MMPs inhibitors. These two types of agents are far from satisfactory and only a few months of survival benefits have been achieved generally. In order to make definitive breakthrough from this stalemate, novel ideas and some even shotgun-like molecular expeditions of drug that is therapeutically related with metastasis itself seem to be a future solution.[5] One of these novel targets has been aberrant sialylation in neoplasm tissues.[6-8] It is not a well-studied therapeutic target. In this review, an important target at sialylation alterations in neoplasm tissues has been documented, described, discussed and highlighted in order for readers to catch a clearer glimpse of it.

Sialic acids (Sias, neuraminic acid) are a special series of 9-carbon backbone acidic carbohydrates and typically found at outermost part of sugar chains attached to cell membrane macromolecules. They play many important roles in a series of physiological and pathologic processes, including microbe binding that leads to infections, regulation of the immune response, the progression and spread of human malignancies and in certain aspects of human evolution.[9-11] The earliest work tackling the phenomenon of a positive relationship between sias and tumors can be traced back to Kimura et al. from 1958.[12-13] Their discovery is tumor cells might excrete and contain more sialyl glycoproteins or glycolipids. These characteristic later have been found to link with highly metastatic tumor types.[14] It has been shown that there are higher sias contents in highly metastatic tumor cell lines than those in lower metastatic tumor cell lines. Since then, numerous similar reports and reviews have been published rapidly. Novel ideas can be mainly concluded as follows.

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### Patients with tumors of high levels of sialyl antigens appear to be linked with poor prognosis of patients

Many researches have showed patients with tumors of high levels of sialyl Lewisx antigens appear to be linked with poor prognosis of patients,[15-16] which is one of the most conspicuous pathologic features of sias in tumors clinically. Since this sialyl Lewisx antigen might be tumorigenic and leads to pathologic outcome to patients, monoclonal antibodies or small molecule chemicals against these antigens might be potential therapeutic agents. These researches need to be strengthened.

### Cancer patients can carry tumors of different sialyl profiles and sias analogues

More than 40 different types of sias monosaccharide's have ever been discovered,[17] which can be linked with other normal monosaccharide (heptose or hexose and so on) to form tremendously diversified 2-6 sugar component antigens (sugar chains) - sias are often at the farthest end of antigens and glycoproteins. Among these antigens, some of them are very tumorigenic and widely occurred among different tumors, such as sialyl Lewis X and A is known to correlate positively with colon and non small cell lung cancer and core α6-fucosylation with liver and pancreatic cancer.[9] These diversified antigens feature mainstreams of current efforts.[9,18] It has especially etiology, pathology, diagnostic and therapeutic forecast values. Any drugs or monoclonal antibodies can be developed to target these anti-

gens for the treatments of relevant tumor types.

## Human sialyltransferases and sialidase as cancer markers and drug's targets

To consider the possible routes for tumor cells to accumulate sias, one might immediately relate them with enzymes. Human sialyltransferases and sialidase as cancer markers and drugs' targets have also been suggested. All the difference between linkage and substitutions of sias might be the results or aftermath of the activity and contents of sialyltransferases or sialidases changes in tumors. It adds the complexity, volume and intensity of researches. Future new technologies of specific detections of these enzymes and their activities will help us to understand these relations. Now, many sialyltransferases or sialidases have been found to be expressed relatively higher or lower in tumors than in normal tissues.[19-22] Cancer stem cells or cancer stem-like cells that are especially important with resistance to chemotherapy, radiation and neoplasm metastasis are important models for pathologic and therapeutic study of sias in tumors. Since sialyltransferases and sialidases appear to have important functions in normal stem cells as well as in development. The differences of sialyltransferases and sialidases manifestations between these normal cells and stem cells can help us better understanding sias biology and pathology in stem cells. This is an important route to explore agents targeting these pathways. This could be very useful in cancer treatments, such as diagnosis, therapeutic targets and basic oncology studies. For example, we can diagnose early cancer by determining sialyltransferases and sialidases levels and activities of patients.

## Cancer-related biology of sias-regulations and diversify

We have entered a new era of applicable stage from an age of merely discoveries of natural phenomena of sias into a well-foundation discipline. In order to make new ages of applications, we must first know the rules of sias in nature. Two important areas are especially intriguing.

Since sias consist of more than 40 sias sugars (monosacchrides),[17] what is their similarity and differences? Is there some cancer-specific analogues existing? There was once an argument that N-glycolylneuraminic acid (Neu5Gc) is cancer-specific in humans.[23-27] We have discovered that NeuGc has higher biological activities than naturally most abundant sias analogue 5-acetyl-neuraminic acid (Neu5Ac, NANA) at equal molar concentrations (Our preliminary data). Some other researchers also reported changed activity between De-N-acetylneuraminic acid containing gangioside than acetylneuraminic acid containing gangiosides in cancers.[28-30] It may be proposed that accumulation effects of each sias and their recombination in glycans will decide the biologic characteristics of cells that occupy these glycan and further decide the characters of these cell types (normal or malignant). So this problem is an interesting, important and unresolved challenge.

Since all the reasons we give above, we must pay more attentions to the regulation and functions of sias in cell, especially in cancer cells. All the difference between linkage and substitutions of sias can be recorded by glycome. Glycomes of sialylations mainly consist of lectin-binding and/or antibody-binding techniques or modern chromatography combined with Mass-spectrometry.[31-33] They are far advanced than colorimetry techniques. Colorimetry can only detect total sias contents. However, an HPLC method combined with a fluorescence detect can also detect sias analogues.[25] Since more than 40 types of sias have been discovered, they are evolutionary and oncology related. Future new technologies will help us to understand these diversifications in quicker and easier ways. Glycome and proteomics, immuno-histological tools and HPLC etc will give us more detailed information about sias in tumors than ever before.[34-35] It might give birth to new round of investigations for therapeutics study different from those in last century. It could determine how many (such as 1-4 constitution of sias) and what sialyl profiles in individual tumor glycoproteins. Apart from that, some other technology and genetic means currently negligible, such as epigenetic considerations of sialylations in tumors might also be some important topics in future.

## Experimental therapeutic study

All these reports of cancer biology or pathophysiology characteristics linking glyco-epitopes indicate their significances in cancer therapeutics. The therapeutic studies of sias-related antimetastatic drugs began at a quarter century ago.[36-38] Since this type of anticancer drugs (especially targeting sias in tumors) has been seldom entering into clinical investigations, only experimental therapeutic studies have been commonly reported. We conclude as follows:

In the initial stages of therapeutic study, the novel antagonists had sometimes been sias derivatives, conjugates and polysaccharide.[36-40] For example a sias-conjugator has been reported to inhibit pulmonary metastases of a mouse colon adenocarcinoma.[36-37] These data have been done in vitro or in mice and there have been no systematic clinic data. We must emphasize and encourage more these study into clinics. These agents have been found to be almost no toxicity to humans, only low hepatotoxicity of sialyl polysaccharide after long-term application has been found in 1st phase clinical study.[39] A disaccharide precursor of sialyl Lewis X, though links with biology of cancer malignancy, can inhibits tumor metastasis *in vitro*.[40] It imply that sialyl Lewis X antigen can have feedback activity in the cell carcinogenesis or metastasis.

Since many sias-conjugators or sialyl antigen can inhibit tumor metastasis, sias-conjugates can be regarded as potential therapeutic agents to treatments of neoplasm metastasis. We can synthesize a series of similar compounds to be tested as anticancer or antimetastatic drugs and further study their anticancer and antimetastatic mechanisms. E.g. where do sias-conjugates or antigens target in tumor growth or metastasis? They may most possibly act in those metastatic pathways that sias participate. It must be novel therapeutic targets waiting us to clarify.

To evaluate a possibility of sias in tumors as anticancer or antimetastatic target, we have carried out a large-scale pharmacologic evaluation in mice for building the relation between sias inhibitions and treatment outcomes of drugs. Our experiment was to study if anticancer (especially antimetastatic) drugs can inhibit sias levels in mice bearing tumors.[25,41] Our work shows that some of anticancer drugs, especially antimetastatic drugs, including Pro and nitrogen mustard, can significantly inhibit serum sias levels in mice bearing tumors S180 and Lewis lung carcinoma. Other sporadic reports have also shown the sias concentration inhibitions by drugs in mice bearing high metastatic tumors B16-F10 [42-43] (Table 1-2). Our work also showed it is not every first-line anticancer drug can inhibit sialic acid levels in mice bearing tumors. Many cytotoxic agents that target tumor metabolism showed no inhibition on serum sias levels in mice bearing tumors. It is those that show antimetastatic activity will more possibly inhibit serum sias level in mice bearing tumors.[41] Similar to our standpoint, Abde-Hamid NM et al. reported that some anticancer drugs that did not show typical antimetastatic effects, such as 5-Fu, could not inhibit sia levels in tumor cells.[44] These work show the inhibitions of sias in tumors can be a good model to study antimetastatic drugs than antiproliferative drugs and be significance for studying their underlying mechanisms. This is a relatively new therapeutic target for us to study.

Chiang *et al.* reported a novel sialyltransferase inhibitor AL10 inhibits adhesion, migration, actin-polymerization and invasion, however AL10 has no antiproliferative effect on cancer cells.[45] This coincidence with previous pathologic view - sialyltransferases and

sialidases are important in neoplasm metastasis. In future, more sialyltransferase or sialidase inhibitors may be tested for their antimetastatic efficacies. It is also a new therapeutic target. However, there are many sialyltransferases in normal and cancer tissues. Currently, the understanding on these diversified distributions and functions of sialyltransferase between normal and malignant cells is far from completion. But this may be a shortcut to reach our goals of development of new antimetastatic drugs after our understanding these differences of sialyltransferases between normal and malignant cells.

Moreover, when sias are chemically introduced into structure of anticancer prodrug, the prodrug uptake by cancer cells and cytotoxicity of the prodrug increase.[46] So, the sias can be used as part of drugs, their function is to increase the drug distributions specifically in metastatic foci and increase the efficacy of anticancer drugs.

## Future directions

We have offered a quick glimpse of the critical issues of relationship between cancer and sialylation aberrant in tissues in this article. Since there are obvious differences of sias monosacchrides or sialyl antigens in different tumor cells, glycome can detect these differences of sialyl antigens and so on. So we can understand better about aberranty sialylation in tumor growth and metastasis by glycome study. Sialyl antigens are important biomarkers in tumor cells and can be treated by targeted monoclonal anitibodies. This is one type of personalized medicine.[47] Diagnostic means leading to diagnosis of cancer biomarkers and providing useful information for individualized cancer chemotherapy is useful areas for sias research. On the other hand, since sias are largely present sugar components, they play diversified physiologic and pathologic functions in large population of living bodies,[9-11] their therapeutic drugs might lead to other biology effects. It adds much more complexity and mystery in current perspective. As the *central dogma* of glycobiology is still unknown,[10] some fundamental questions related to carbohydrate itself are even more welcoming and decisive to our understanding to nature of neoplasm metastasis and their inhibitions by drugs.[10,48] We foresee a good future waiting for us if we insist on these researches.

## Conclusions

There are plenty of questions to be asked and answered relationship between pathology and therapeutics and relationship between sias and tumors. This needs times and fortunes and high-talented scientists and assistant personnel. However, this is high possibility of success. In this critical time, we ought to consider changing our focus a little bit from current stalemate of angiogenic therapy into some other newer approaches such as fibrinogen-pathway or cancer cell movements.[49-50] Aberrantly sialylation in tumors seems to be a new start and shortcut. Why don't we wait and see the results?

**Table 1. Glossary of drug inhibitions on serum sialic acid levels in mice bearing tumors.**

| Tumor cell lines | Drugs | Detective methods | Authors |
|---|---|---|---|
| S180; Lewis lung carcinoma | Antineoplastic drugs | Colorimetry | Lu DY, *et al.*[41] |
| S180; Lewis lung carcinoma, Hep A | Antineoplastic drugs | HPLC | Lu DY, *et al.*[25] |
| B16-F10 | Sulforaphane | Colorimetry | Thejass P, *et al.*[42] |
| B16-F10 | Oligonol | Colorimetry | Lee SJ, *et al.*[43] |

**Table 2. The influence of Sulforaphane on serum sialic acid level in mice bearing B16F-10.**

| | Schedule | Sialic acid μg/mL |
|---|---|---|
| Control (normal mice) | | 21.3±1.5 |
| Tumor-bearing mice | | 108.26±1.92 |
| Sulforaphane | Simultaneously | 35.13±0.9 |
| Sulforaphane | Prophylactic | 59.51±1.2 |
| Sulforaphane | Developed metastases | 92.88±1.23 |

Adapted from Thejass P, *et al.* work.[42]



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