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Review

Harnessing cancer cell metabolism for theranostic applications using metabolic glycoengineering of sialic acid in breast cancer as a pioneering example

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ABSTRACT

Abnormal cell surface display of sialic acids – a family of unusual 9-carbon sugars - is widely recognized as distinguishing feature of many types of cancer. Sialoglycans, however, typically cannot be identified with sufficiently high reproducibility and sensitivity to serve as clinically accepted biomarkers and similarly, almost all efforts to exploit cancer-specific differences in sialylation signatures for therapy remain in early stage development. In this report we provide an overview of important facets of glycosylation that contribute to cancer in general with a focus on breast cancer as an example of malignant disease characterized by aberrant sialylation. We then describe how cancer cells experience nutrient deprivation during oncogenesis and discuss how the resulting metabolic reprogramming, which endows breast cancer cells with the ability to obtain nutrients during scarcity, constitutes an "Achilles' heel" that we believe can be exploited by metabolic glycoengineering (MGE) strategies to develop new diagnostic methods and therapeutic approaches. In particular, we hypothesize that adaptations made by breast cancer cells that allow them to efficiently scavenge sialic acid during times of nutrient deprivation renders them vulnerable to MGE, which refers to the use of exogenously-supplied, non-natural monosaccharide analogues to modulate targeted aspects of glycosylation in living cells and animals. In specific, once non-natural sialosides are incorporated into the cancer "sialome" they can be exploited as epitopes for immunotherapy or as chemical tags for targeted delivery of imaging or therapeutic agents selectively to tumors.

1. Introduction

Cancer is a leading cause of mortality in developed nations (in several regions of the USA it has overtaken cardiovascular disease as the leading cause of mortality) and is threatening to become the planet's number one killer in the next generation [1,2]. Despite extensive efforts over the past several decades dating from the declaration of President Nixon's five year war on cancer declared, survival rates for this disease have barely improved. Halting progress in the war against cancer has heightened urgency for new approaches for identifying biomarkers and developing new treatments [3–5] that will allow (1) better prediction of cancer risk; (2) earlier diagnosis; (3) classification of the degree of malignancy to facilitate individualized, patient-appropriate therapy; and (4) methods to monitor therapeutic effectiveness. Ideal biomarkers will be readily translated into the clinic, be easily combined with *in vivo* cancer imaging technologies [6], and will be effective at discerning the metastatic spread of cancer, which results in the formation of aggressive secondary tumors in distant organs that ultimately results in the death of most cancer patients [7–9].

This report describes prospects for how cell-surface

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glycosylation, which has long been recognized as distinguishing feature of cancer in general, can be exploited for the discovery and exploitation of new and improved biomarkers and therapeutic options with a focus on breast cancer for two reasons. First, breast cancer remains a leading cause of death for women worldwide and especially in developed nations [10]. For example, in 2012, there were 1.7 million new breast cancer cases representing about 12% of all new cancer cases and 25% of all cancers in women worldwide with the highest incidence rate in North America with ~92 cases per 100,000 people in the United States and 80 in Canada. The second reason is that breast cancer has been used to pioneer the study an underappreciated aspect of glycan biosynthesis, which are connections between glycosylation and abnormal glucose metabolism now broadly associated with many (or most) types of cancer. These provocative findings suggest that nutrient deprivation illuminates and exacerbates glycosylation changes in cancer and can provide a foundation for new diagnostic and therapeutic approaches.

Connections between nutrient availability, metabolism, and carbohydrate structures that serve as putative cancer biomarkers are a major focus of this article, we first (in Section 2) provide a general overview of glycosylation in cancer and then (in Section 3) spotlights sialylation and *O*-GlcNAc, which are aspects of glycosylation that are particularly sensitive to the nutrient status of cancer cells. Subsequently, the role of nutrient deprivation and metabolic flux of nutrients in the *in vivo* cancer microenvironment will be described accompanied by prospects to exploit metabolic glycoengineering techniques to discover, identify, and image biomarkers and develop new diagnostic and therapeutic approaches for breast cancer (in Section 4).

2. Glycosylation in breast cancer

2.1. Abnormal glycosylation is a ubiquitous feature of cancer

Abnormal glycosylation is long-standing feature of cancer. Aberrations in glycan display in cancer were described more than four decades ago when the Hakomori laboratory linked altered glycosphingolipid profiles with metastatic cancer [11–15]. Extensive documentation of carbohydrate structures associated with cancer – often referred to as "tumor-associated carbohydrate antigens" or TACAs for short – was available by the early 1990s [16–18]. Since then ongoing efforts to catalog TACAs across cancers of various tissue origins and stages have shown general trends as well as, in many cases, nuances that hold promise for highly specific diagnosis and therapy. In this report we discuss general features of abnormal glycosylation found in cancer and use breast cancer as a specific example where TACAs play a major role in malignancy and oncogenic progression [19].

Tumor-associated carbohydrate antigens occur at varying degrees of abundance and distribution across normal, benign, and malignant tissues [19–22]; successful biomarker discovery depends on the capture and identification of these TACAs in an efficient and meaningful manner followed by correlation of these levels with the stage of cancer progression. The complexities of glycosylation make the discovery and validation of TACAs a challenging task based on several factors, a few of which are mentioned here. First, the target glycans are not primary, template-based gene products but rather depend on the under-, over-, or neo-expression of glycosyltransferases, glycosidases, and other relevant glycan-processing enzymes; as a result TACA production cannot be predicted in a rigorous manner but in some cases can be estimated from mathematical modeling approaches [23,24]. Second, multiple glycans can decorate a single site on a protein backbone (e.g., a "glycosite" as explained in detail elsewhere [25]) and distinct glycans can be embedded within glycan chain backbone (e.g., "LacNAc" or Lewis X). Finally, although glycans are typically regarded as post-translational modifications (PTMs) of proteins, the sugar structures themselves can be post-synthetically modified (e.g., via acetylation, phosphorylation, or sulfation), thereby increasing chemical diversity and complicating analysis.

The identification of TACA biomarkers has accelerated in recent years by improvements in "glycoscience" technologies – especially analytic methods to identify and characterize glycans from biological samples – that have helped to shed light on how glycans are differentially expressed in cancerous cells, tissues, and body fluids compared to their healthy counterparts [26–34]. Accordingly, it is now recognized that a distinguishing feature of breast cancer is an altered glycan profile that includes: 1) synthesis of truncated glycans (Fig. 1a), 2) exposure of Lewis antigens (Fig. 1b), 3) increased branching of N-linked glycans and 4) changes in sialylation (Fig. 1c). This information has made it clear that in addition to serving as correlative biomarkers of breast cancer, TACAs play active roles in driving breast cancer progression [23]. Examples of the roles of glycosylation in cancer, which are described more detail throughout this report, include regulation of cell surface signaling (e.g., EGFR [35]), facilitation of metastasis [20], and systemic effects resulting from nutrient deficiency (e.g., cachexia [36] or secreted glyco-genes (e.g., sialidases [37]). The inherent complexity of glycosylation pose many daunting challenges when attempting to understand the wealth of nuances introduced in TACAs during oncogenic transformation but at the same time offer unparalleled opportunities for the discovery of unique biomarkers.

2.2. Sialylation plays a dominant role in diversifying breast cancer TACAs

Cell surface glycans in human cells are generally assembled from nine different monosaccharide "building blocks" (as described in detail elsewhere [38]). The addition of one of these sugar moieties, sialic acid – an unusual nine carbon monosaccharide – to the terminal monosaccharide of a glycan is referred to as "sialylation." This widespread and highly heterogeneous modification has been particularly strongly tied to many aspects of breast cancer progression; indeed common TACAs associated with breast cancer are almost always sialylated (Fig. 1). Overall, the several dozen biochemical reactions that determine sialylation in human cells (Fig. 2) provide many opportunities for "something to go wrong" in disease; indeed, dynamic changes in the sialylation of glycans have been observed in many disease states beyond cancer ranging from obesity to neurological disorders to muscle disease [39–42].

Human cells diversify presentation of sialic acid on glycoconjugates in multiple ways that tune biological activity. Heterogeneity arises from several factors beginning with the "site occupancy" of terminal galactose or GalNAc residues that serve as sites for sialylation; as few as 5% (or less) and as many as 90% of such sites – even when produced by a single cell type – can be decorated with this sugar [43,44]. Additional structural diversity is provided by the glycosidic linkage to the underlying Galβ1,3(4) GlcNAc/Glc penultimate glycan termini. For glycoproteins, a single sialic acid typically is attached to the underlying glycan structure through either an α2,3- or α2,6- bond (Fig. 2); in glycolipids an α2,8-linked sialic acid can be attached to an underlying α2,3-linked sialoside as exemplified by the conversion of ganglioside GM3 to the breast cancer marker GD3 [45]. In specialized cases that are highly restricted in healthy adult tissue, an α2,3-linked sialic acid can be extended with multiple copies of this sugar sequentially attached to each other through α2,8-bonds, forming polysialic acid. Polysialic acid (PSA), which is associated with several types of cancer [46–51] including highly malignant breast tumors [49,52].

In addition to the presence or absence of a sialic acid moiety and