opened doors to study such outliers including amide oxidizing F_{420} oxidases, repeats of truncated dehydratase enzymes, and potential leader peptide-like regulatory genes. Conceptually, this work bolsters the current focus in natural products to study the systems of bacterial gene expression to push chemical diversity and the discovery of novel natural products.

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Glycan Microarray Reveal the Sweet Side of Cancer Vaccines

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Advances in genomics and bioinformatics facilitated identification of tumor-specific neoantigens as optimal targets for cancer immunotherapy. In this hot topic, most efforts focus on mutant peptide antigens, overlooking tumor-associated glycosylation changes. Given the latest progress in glycomics, in this issue of *Cell Chemical Biology*, Xia et al. (2016) use glyco-antigen microarrays to investigate immune responses to whole cancer vaccines and provide important insights into vaccine efficacy.

All living cells have a dense layer of cell surface glycans (sugar chains) that play important functions in almost all biological pathways encompassing structural, modulatory, and various immune recognition roles. Glycosylation is as essential to life as the genetic code yet it is quite challenging to investigate, because the glycome is much more diverse and complex compared to the genome, transcriptome, or proteome (Varki, 2016). Cell surface glycans are often altered during cellular development or in various disease conditions, including cancer, and therefore serve as important targets for diagnostics and therapy (Amon et al., 2014).

Glycotherapy is a promising research frontier driving efforts towards glycan-based technologies and treatments, largely attributed to the ability to study and produce structurally defined carbohydrates (Hudak and Bertozzi, 2014). Technical progress in the field allowed production of various glycosylation probes (e.g. inhibitors, imaging probes, glycoengineering extracellular probes), synthesis of homogeneous glycosylated-biotherapeutic antibodies, and development of carbohydrate-based vaccines against cancer (Krasnova and Wong, 2016). Current anti-cancer vaccines usually utilize selected tumor-associated carbohydrate antigens that are specific or highly enriched in cancer cells. Such vaccines require optimization of the carbohydrate-antigen chemical conjugation to selected carriers, as well as their adjuvant and administration protocol, albeit such vaccines have shown limited success so far (Krasnova and Wong, 2016). More recently, therapeutic whole cell cancer vaccines regained attention as a promising active immunotherapy approach in light of their potential to induce activation of the patient's own immune system against tumor-associated antigens to reduce tumor escape. Yet these efforts largely ignored the contribution of tumor-associated carbohydrate antigens in those vaccines.

Glycan microarrays have emerged as a technology that can mimic glycan expression on cell surfaces and provide insights into how glycans function in recognition and signaling within and between cells, viruses, antibodies, and various glycan binding proteins

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(Cummings and Pierce, 2014). In this system, various glycans are immobilized on surfaces in a chip format and their recognition measured in a high-throughput manner. Progress in this technology most importantly stems from improved glycan synthesis and library preparation, as well as array fabrication, detection methods, and assay design (Rillahan and Paulson, 2011; Geissner and Seeberger, 2016). Glycan microarrays are becoming robust and unique tools in biomarker discovery and vaccine development because they can provide important and detailed information of the resulting immune responses against various carbohydrate antigens (Geissner and Seeberger, 2016). Some examples for vaccine-related applications of glycan microarrays include glycan epitope mapping, determination of immunogenicity, characterization of the humoral responses, and evaluating treatment success and variability of responses (Geissner and Seeberger, 2016).

In this issue of *Cell Chemical Biology*, Xia et al. (2016) address an important aspect of whole cell cancer vaccination considering responses to glycans and their effect on patients' outcome. Using a comprehensive glycan/glycoprotein microarray tested at physiological temperature, they demonstrate that the whole cell pancreas cancer vaccine GVAX induced IgG and IgM responses to several targets in human patients with follow up at week 40 and week 48 after immunization. Interestingly, they found that chemoradiation therapy before week 40 had not affected anti-glycan antibodies repertoires, with some targets showing elevated responses, including to tumor-associated carbohydrate antigens. They also found increased responses to non-human glycans and glycoproteins and focus on investigating response to bovine fetuin and α-Gal that was represented by several different array components. These responses were then attributed to non-human products used to produce the GVAX vaccine (e.g., FBS) supported by inhibition of antibodies binding with FBS. These findings highlighted the importance of the production platform itself during vaccine design that could potentially contribute to the response to the vaccine. In this context, manufacturing of other glycosylated biotherapeutics could potentially be affected by materials used in the production line, likely incorporating α-Gal as well as other xenogenic carbohydrate antigens that eventually could affect their efficacy in patients (Amon et al., 2014). In line with that, in their subsequent analysis to evaluate correlation between selected responses with patients' survival, they found that only several IgG responses (but not IgM) supported survival, in particular those against selected α-Gal containing targets. They further found that the α-Gal responses inversely correlated with anti-Galectin-3 responses, providing a potential mechanistic explanation for these antibodies role in patient survival. Importantly, these data were deposited in a dedicated database and could be accessed by others who may be interested in more in depth analysis of other targets not fully described by Xia et al. (2016).

The concept of using glycan microarrays as a means to investigate and evaluate vaccine responses is emerging as an important area of research because of recent significant advances in carbohydrate synthesis and isolation of selected glycan species and is expected to grow as more comprehensive or specifically designed arrays are developed. In that sense, it is also obvious that as glycan microarray data accumulates, a centralized standardized database needs to be established so that the results from different laboratories can be compared and integrated. Given the natural complexity and diversity of glycans, it also becomes more and more clear that presentation modes of glycan antigens, their spacing, length, flexibility of glycans and their carriers, as well as glycan surface organization in multi-complex units or patches, all contribute to an even higher level of complexity and fine-tuning of their immune recognition (Kiessling and Grim, 2013; Varki, 2016). In keeping with this, altered expression of cell surface glycans is attributed to changes to the normal metabolic pathway and involve altered expression of various glycosyltransferases, glycosidases, sugar transporters, and the availability of the carbohydrate building blocks. Systems-level analyses of glycan diversity management allow integration of genome, transcriptome, proteome, and metabolome to guide selected design of glycosylation patterns on cells (Spahn and Lewis, 2014), and together with the advent of powerful high-throughput glycan microarrays and specific glycan detection tools, would facilitate a more complete view of the biological role of glycans and their immune recognition.

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