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News & Views

Title: Enhancing the glycosciences toolkit: new GAGs in the lineup

Sulfated glycosaminoglycans (GAGs) are polysaccharides which are the most complex glycans in nature, with roles in a wide range of crucial biological functions that are also relevant to many disease processes. Their complex patterns of sulfation permit them to bind and regulate cognate proteins, and repesents a kind of molecular code that underpins their selective modes of action. However, determining the structure-function relationships of this code has remains a significant challenge to progress in the field, due to the inherent difficulties in analysis of these glycans and the lack of analytical tools. Now two labs have harnessed gene editing techniques to engineer stable changes in the cellular machinery that produces GAGs[1,2], in particular exploiting recently developed clustered regularly interspaced short palindromic repeat (CRISPR)-CRISPR-associated gene 9 (Cas9) as a rapid and relatively simple route to stable knockout cell lines. In doing so they have created new cell variants which produce divergent and novel GAG structures that represent a rich resource to propel advances in the field.

Chen et al[1] developed a cell library resource described as the "GAGome" using widely exploited Chinese hamster ovary (CHO) cells as their model system. Using CRISPR-Cas9 they developed a validated set of guide RNAs (gRNAs) and employed these to knock-out (KO) specific combinations of genes, along with some selected gene knock-ins using established zinc finger nuclease (ZFN)-based approaches. This permitted large-scale combinatorial gene engineering of a wide variety of genes in the biosynthetic pathway for GAGs of both the chondroitin/dermatan sulfate (CS/DS) and heparan sulfate (HS) families. In contrast, Qiu et al[2] focussed on the HS family genes in primary lung endothelial cells, exploiting Cre-Lox recombination technology and a number of existing mutant mice strains (as well as additional application of CRISPR-Cas9 in some cases) to derive a diverse library with combinatorial KO of expression of key biosynthetic and remodelling enzymes.

In previous work mutant CHO cell lines derived by classical chemical mutation methods have been a powerful tool[3], along with more recent availability of KO cell lines derived from transgenic mice (with mainly single or dual combinations of altered enzymes)[4]. Combinatorial application of the small interfering RNA (siRNA) approach in cell lines has also been usefully employed[5]. These certainly have experimental value but also limitations in scope. In contrast, the advantages of the new platforms are the ability to produce systematically engineered isogenic variants on larger scale, and in a wider variety of cell types, thus dramatically expanding the potential scale of such methodologies (see Figure).

The new cell libraries were validated by showing how combinatorial mutants could effectively tackle key questions in the field. Firstly the cells provide the basis for a powerful new genetic dissection strategy to explore the biosynthetic and remodeling processes involved in GAG production; in concert with structural analysis of GAG disaccharide composition they provided further insights into the complex inter-regulation of the network of enzymes involved[1,2], including effects on chain length[2]. Secondly, the cells were used in binding experiments to show that they could be used to explore the specificity of GAG-protein interactions, exemplified by growth factors, pathogen receptors and antibodies[1,2].

Furthermore, the functional impact of the engineered changes could be examined in terms of endogenous signaling responses to, for example, growth factors[2]. Importantly, these data provided further evidence that structure-activity relates to HS fine structure and not just sulfation levels, and that nuanced relationships exist between binding and functional effects[2]. Finally, it was demonstrated that the engineered cells could be used to produced novel products with defined GAG chains, including recombinant proteoglycans, or secreted GAG chains "primed" with xylosides (as a proxy for the natural core protein)[1]. The latter could also provide a chemical tag at one end of the chain which could be exploited in the future to permit their oriented attachment to surfaces including glycoarrays[1].

These new technologies now provide almost boundless opportunities for expanding the libraries and exploiting them in a broad range of applications (see Figure). Crucially, these tools will appeal not only to existing glycobiology aficionados but will surely open up the field to wider life and biomedical sciences researchers previously reluctant to grapple with the complexities of GAG functions, despite their evident importance. In particular the use of CRISPR-Cas9 with validated sets of gRNA targeting constructs[1] will enable studies in virtually any cell type in the future. The opportunities for cell–based screening extend beyond using the cells in binding studies, since they will allow extensive dissection of structure-function relationships in specific cell contexts. They can also be exploited to better define bioactive GAG motifs of biological relevance (especially domain structures reflected by longer sequences of disaccharides), to address key questions about selectivity of GAG structures for protein interactions, and also regulation of the biosynthetic network. A key opportunity will be to better understand the role of 3-O-sulfation of HS, a rare event which involves a family of at least seven enzymes but whose functional role is currently poorly understood.

The cell lines also provide a rich new resource for creating novel GAG-based compounds, including content for populating microarrays with defined GAG chains to measure binding capacities[6] and potentially live cell signaling responses[7]. The derived GAGs could also be used to generate defined bioactive saccharide libraries[8] for screening in biochemical and bioassays relevant to many different biological processes. Collectively the exploitation of these resources should yield a step-change in insights into the structure-activity relationships of GAGs, and could underpin identification of new drug candidates. Finally, the cell libraries will also provide a potentially scaleable "recombinant" source of novel defined GAG chain and PG biomaterials for applications including regenerative medicine[9].

Together with ongoing advances in mass spectrometry techniques for detailed sequencing of bioactive GAG domains[10] these cell-based libraries and derived resources hold huge promise to alleviate the current bottleneck in our understanding of selectivity in GAG-protein interactions. This can ultimately be expected to underpin major new advances in biomedical and biotechnology applications of GAGs as a novel class of therapeutic entities.

(980 words)

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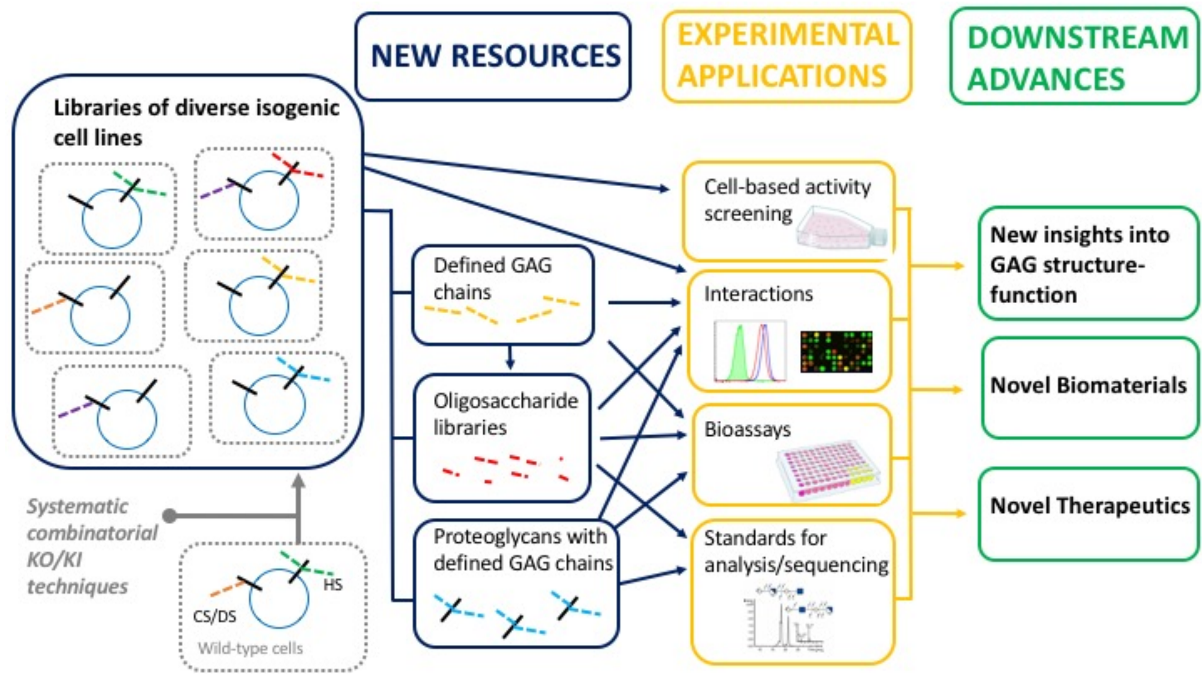
Figure legend: Systematic cell-based GAG libraries and related resources: exploring specificity and generating novel bioactive entities.

Recent gene editing techniques can be leveraged to engineer libraries of cell lines with stable changes in the cellular machinery that produces complex polysaccharides of the glycosaminoglycan (GAG) family. This provides a rich new resource for exploring their structure-activity relationships in diverse biological processes underpinning many diseases. Exploiting these libraries to the full holds the potential to create a step-change in the field and drive advances in downstream applications such as discovery of novel drugs and biomaterials.


NEW RESOURCES
EXPERIMENTAL APPLICATIONS
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Libraries of diverse isogenic cell lines
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HS
Wild-type cells
Defined GAG chains
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Proteoglycans with defined GAG chains
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