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# Glycosaminoglycans (GAGs) in Cardiovascular Disease: Searching for the Sweet Spot

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## Editorial

*Dulcet notes sound,*

*Round tense cables,*

*Ringed bridges and ridges,*

*Surround,*

*Complex and stable,*

*Sweet spans of time,*

*Steel lattice of grace,*

*Linking all space,*

*Suspended in line,*

*Rising towers,*

*Sweet essence of power.*

(Alexandra Lucas, MD, 2016)

One of the seminal discoveries in biology was the discovery of the structure of deoxyribonucleic acid, DNA, by James Watson, Francis Crick and Rosalind Franklin that led to the later deciphering of the genetic code by a series of exceptional researchers. Without Rosalind Franklin's X ray crystallography work on the hydrated form of DNA, Watson and Crick would not have been able to decipher the double helix [1]. It is this underlying structure that allows DNA to direct the transcription of RNA and later translation of the genetic code into proteins. In simple terms without understanding the structure, the genetic function of DNA was not evident. While the genetic code directs all protein synthesis, it is also true that in many instances understanding what genes are expressed and translated does not inform the actual final functions, the end result of protein expression and activity. The mere presence of a protein or enzyme also does not translate directly into an outcome in activity. Function is a much more complex process. Thus the genetic code can direct amino acid sequence and structure, but structure and location can also modify ultimate function. With this editorial we discuss the effects of one structural component, the glycosaminoglycans (GAGs), on the functional activities of other molecules, cells and organs. GAGs represent one of the silent engineers of cellular and tissue structures. There has been an intense and fascinating focus on genetic analysis and in fact all OMICs, genomics and proteomics, analyses but simple listing and alphabetizing of what is expressed does not provide a complete understanding of how this leads to cell and tissue actions.

The ability to decipher the meaning of nucleic acid sequences and how genes are transcribed to messenger ribonucleic acids (mRNA) and then on to translation into amino acid sequences within proteins, revolutionized the biological sciences. More recent work has led to deciphering of whole genomic sequences in many organisms from viruses, bacteria, plants, insects and of course, mice and men. However, one of the most daunting tasks is to examine the true functional consequences of gene expression from the microRNA and long non-coding RNA epigenetic modulation of gene expression to the actual regulation of downstream protein expression and activity. There is a well-known lack of correlation between mRNA and protein expression in many cellular contexts. For example, it has been demonstrated that a single cell's mRNA copy numbers and protein are uncorrelated for any given gene [2]. Thus, expression of a gene into messenger RNA and eventually protein does not necessarily translate into insights into biological activity, as each protein or enzyme can be further modified by events such as assembly into quaternary structures, cleavage, activating and also degrading proteins all of which modify protein levels and functions. Protein activity is also modified by co-factor elements such as calcium and magnesium or addition of sugar residues, as in glycosylation. Some of these elements have independent functions, working outside of protein and enzymatic functions, e.g., in simple terms acting on their own. Thus, much as we would like for there to be a direct one to one, left brain relationship of gene expression and protein activity, the activity of proteins is extensively modified by later post-translational processing. In simpler terms we cannot fully predict the outcomes of gene expression by analysis of genomic sequences alone.

For proteins, there have been many reports of independent functioning domains and metabolites cleaved off from parent proteins. For example the apolipoprotein E (ApoE) protein with cholesterol transport activity also has anti-inflammatory functions in select domains as does the calcium regulating protein calreticulin. Angiotensin II is a highly active vasoactive protein that is cleaved from a parent angiotensinogen protein. Angiotensinogen is a serine proteinase inhibitor or serpin that in fact lacks normal serpin protease inhibitory activity but gains activity when cleaved. Other serpins such as alpha1 antitrypsin (AAT) and heparin cofactor II (HCII) have functional peptide metabolites with anti-viral and anti-endotoxin

activity [3]. We have also recently examined peptides from the naturally cleaved reactive center loop (RCL) of a virus derived serpin, Serp-1. Serp-1 is a virus derived protein encoded and expressed by Myxoma virus as a defense against host immune myeloid cell actions against viral invasion. Serp-1 inhibits the thrombotic and thrombolytic proteases and blocks inflammatory cell activity both in viral infections and also in animal models of vascular disease and transplant when given as a purified protein. Select Serp-1 RCL peptides have independent anti-plaque, anti-inflammatory activity in aortic transplants in mice [4]. One such serpin RCL peptide also improves survival in a lethal mouse viral infection. None of the extended functions of these proteins was predicted directly from a genetic sequence.

Thus while the nucleic acids build RNA from DNA and then the mRNA builds proteins, providing a simple code to analyze and allowing us to read a path from gene to final protein expression, the analysis of proteins and also other components in cell and tissue structures such as glycosaminoglycans (GAGs) presents a complex and yet powerful force in organismal biology. GAGs are polysaccharides formed by enzymatic polymerization or broken down by degrading enzymes, but the linear and sulfated formations are synthesized by enzymes and do not fit a direct, genetically predetermined length or shape, other than through modifying enzymes. Furthermore the organization of GAGs to form gradients along which inflammatory activated cells can migrate into organ tissues, cannot be predicted from a DNA sequence [5]. The term, "heparanosome", is used by some to refer to the intracellular heparan sulfate synthetic machinery comprised of multiple enzymes localizing to the Golgi apparatus. Thus, HS is synthesized by multiple steps without a one to one relationship to gene expression [6]. The GAGs have long been considered to be adjunct or simple structural reagents, forming a mechanical tissue supporting structure, but recent studies have discovered that in addition to modifying enzyme and protein activity, these sugar polymers are now found to have critical independent functions. We are only beginning to understand the extent of their impact on normal organ, tissue, glycoprotein and protease functions.

In the field of cardiovascular disease, glycoproteins have long been reported to modify atherosclerotic and inflammatory plaque development as for perlecan and syndecan. I distinctly recall one young professor pointing out the connective tissue in spaces between cells and on histology cross sections of atherosclerotic plaque in rabbit models and asking a simple question, 'What is in these connective tissue spaces?' This very astute observation led to his work on perlecan (Brad Strauss, University Toronto, ON, Canada). We know that the glycocalyx surrounds endothelial tissues and other areas in the arterial wall, forming a layer that can activate and inhibit proteins. Heparan sulfate is the predominant GAG in the arterial wall and the absence of HS has proved incompatible with viable function. Heparin is a related GAG that is known for, and has been used long term as an anti-coagulant, to reduce clots. Heparin when used as a drug is in fact produced as an unfractionated mix of multiple length polysaccharides extracted from connective tissues. Heparin is also used as a fractionated mix of more select length polysaccharides. Heparin is widely used to treat clot formation and as an initial treatment for deep vein thrombosis (DVT) and pulmonary emboli (lung clots) which can be lethal. Heparin is also given for acute myocardial infarctions (MIs) as well as for prevention of thrombosis during percutaneous coronary interventions (PCI), and stent implants. Heparin is used to maintain patency of venous access ports for chemotherapy and for AV fistula grafts for dialysis and to maintain blood flow during bypass surgery. The molecular function of heparin is to activate a serpin, called anti-thrombin III (ATIII), increasing ATIII activity by 100-1000 fold. Heparin also modifies function of other serpins such as PAI-1. This adjunctive function of heparin and potentially tissue HS GAG, both as a drug and in arterial tissues, cannot be predicted from a genetic sequence.

Of even more interest, recent work has demonstrated that modifying HS structure through conditional deletion of the sulfotransferase enzyme N-deacetylase-N-sulfotransferase-1 (Ndst1-/-) can alter antibody formation after transplant and even reduce acute nephritis [7-9]. Heparan sulfate (HS) is the predominate GAG in the glycocalyx that surrounds the endothelial cell layer, the inner lining of the arterial wall. Altering HS content and sulfation can increase or inhibit neutrophil adhesion and migration. GAGs also bind chemokines, forming directional platforms for macrophage and T cell invasion into the arterial wall and other tissues. Many inflammatory responses are thus driven by endothelial cell dysfunction and modifications in synthesis and degradation of the glycocalyx [10]. Modified GAGs and metabolizing enzymes, heparanase and chondroitinase, are also variably reported to modify acute kidney damage. We have also found that changes in Ndst1 expression in mouse aortic transplants can reduce plaque growth [11] and more recent work has demonstrated a correlation between changes in HS GAG disaccharide content and reductions in acute rejection in Ndst1-/- donor allograft transplants in mice (Manuscript submitted, unpublished data). What is both fascinating and also supremely frustrating is the fact that one cannot fully predict the functions of HS and other GAGs, such as chondroitin and dermatan sulfate the main connective tissue and glycocalyx GAGs, by a direct correlation to gene expression. Once expressed, the enzymes such as Ndst1 or heparanase function to polymerize and / or to breakdown the GAGs, respectively, with no direct relationship to gene expression. Further, the final effects of gene expression form an excellent basis for discovery, but subsequent protein and GAG expression analysis is required to fully understand the roles of selected genes in functional systems. This analysis often requires whole animal models or even selective conditional gene deficiency in individual cells to determine where and when gene expression is important to later protein expression, GAG polymerization and finally structure and function.

As for the discovery of the helical structure of DNA, things that appear simple on the surface are not always so simple. For example while the discovery of the double helical structure of DNA is generally attributed to Watson and Crick, in reality, and as stated in the definitive book by Watson (The Double Helix), they required Rosalind Franklin's X ray crystallography data to finally solve the puzzle. However, while it is openly stated in this book that the data was accessed without her permission, Franklin is often forgotten, ignored as having made a seminal observation that provided the framework, the basis, for this extraordinary discovery. Indeed similar to the omission of credit for Franklin in the discovery of the structure of DNA, the supportive and structural elements of the glycocalyx and the GAGs are crucial, essential to understanding normal biological function of the cardiovascular system and yet are often ignored and overlooked.

These underlying GAG structures, designed by silent tissue engineers, modify function and their crucial role is thus often overlooked, much as one seldom notices a bridge we use to cross a river. Thus the role of polysaccharides and other connective tissue elements are present, hiding in plain sight, and yet performing not only

supportive but also pivotal actions, changing cell, tissue and molecular functions. Or as quoted by one of us (MB), Jean Paul Sartre once stated "Existence precedes Essence" implying a structure must exist before its function or essence can be divined. When one examines simple yet complex structures such as the glycocalyx in the arterial wall and in the connective tissue, there are additional functions that have, as yet, only been minimally understood and tapped.

We would like to state that it is now time to search for the function, to better understand how supportive glycosaminoglycan (GAG) structures can profoundly modify biological functions. In summary, the GAG family is an engineering marvel supporting, bridging and regulating tissue function, the sweet spot in the connective tissue.

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