Review Article

Understanding the Basic Role of Glycocalyx during Cancer

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Abstract

Metastasis or cancer is a functional, molecular and structural disorder which has been an unsolved and fatal mystery and leads to death in most of the individuals suffering from it in spite of the advances made in biomedical and oncological fields. Structurally a tissue consists of cells enclosed by glycocalyx (partially or completely), extracellular matrix incorporating lymphatic and mircovessels. There is a specific amount of glycocalyx sandwiched between extracellular cell matrix and cell membrane depending on the type of the tissue and cell and their location in the biosystems. The common constituents of glycocalyx include biomolecules such as glycolipids, glycoproteins, and oligosaccharides; the glycoproteins are trans-membrane proteins. Any impact due to the interaction between inter- and/or intra-cellular biomolecules or any expected xenobiotics affect extracellular matrix, glycocalyx, cell membrane, cell organelles; these are the prime targets for the investigation related to metastasis. Somehow or the other the glycocalyx has attracted relative less attention of the researchers. The various aspects of the prometastatic interactions involve ligand-receptors, integrins, and other cellular receptors; glycocalyx has its role in such interactions. There are changes in the physicochemical parameters of glycocalyx which affect the cell membrane adversely. These result in malfunctioning of cell signaling, cell proliferation, cell migration, etc. There have been relatively less reports on the structural and functional changes in glycocalyx specifically related to circulating tumor cells and the cancerous cells of organs such as ovary, breast tissue, lungs, and hepatic tissues. In this presentation, an effort is made to review and evaluate the changes in glycocalyx during such interactions between the glycocalyx and the prometastatic molecules.

Key Words: *Circulating-tumor-cells, glycocalyx, hydrodynamic sheer forces, mechanotransducer, metastasis, transmembrane glycoproteins*

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Introduction

A biosystem is an amalgamation of molecular entities. Its existence is the representation of its amelioratory effects in its specific niche may it be within the organism or in an environment. Cell is the structural and functional unit of a biosystem. The structural and functional aspects of cell and cell as whole, experiences the physical and chemical forces and exhibits respective responses that constitute the cell behavior. These responses are dependent on the time and length scale concept. Cells of biosystem such as plant, algae, fungi or bacteria have flexible or rigid layer consisting of carbohydrate rich zone; this structural aspect is protective, functional and supportive in nature; but in case of animal cells and most of the eukaryotic cells, there is no such rigid cellular enclosure. Instead animal cells have the extracellular matrix. Animal cells and their extracellular matrix share a unique carbohydrate-rich layer that is referred as 'glycocalyx'. It is placed adjacent to plasma membrane. Precisely, a cell membrane is enclosed by glycocalyx externally and cytoskeleton internally. The various functional abilities of a cell are related to the functional abilities of the glycocalyx, cell membrane and the cytoskeleton as one unit.

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Glycocalyx in Biosystem

Structural aspects of glycocalyx

Plant, algal, fungal and bacterial cells have least flexible or rigid layer consisting of carbohydrate-rich zone; this structural aspect is protective, functional and supportive in nature.[1] Animal cells and most of the eukaryotic cells are not provided by such rigid cellular enclosure along with cell wall. Instead animal cells have the extracellular matrix,[2] cells and extracellular matrix share a unique carbohydrate-rich boundary; this boundary is regarded "glycocalyx." It is placed adjacent to plasma membrane. The glycocalyx is differentiated into attached layer and unattached layer. The attached region of glycocalyx has an inherent part pertaining to cell membrane, this region cannot be mechanically separated, if one tries to remove it some part of cell membrane is also gets removed.[1] According to Cooper, structurally the glycocalyx is made of glycolipids,

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glycoprotein and oligosaccharide; the glycoproteins are trans-membrane proteins. The trans-membranes glycoproteins such as selectin-E, selectin-P and selectin-L play an important/essential role and help in recognizing specific oligosaccharides that are present on the cell membrane of the interacting cells. This feature is very common during the adhesive interaction and is highly facilitated by identification of oligosaccharide on the endothelial cells.[3] Among multicellular organisms the oligosaccharides act as "cell-markers" to recognize the type of the cell. Trans-membrane glycoproteins of endothelial cells and white blood cells get interlinked involving fucose, galactose, N-acetylglucosamine, and N-acetylneuraminic acid also referred as sialic acid.[3]

Reitsma *et al*. reported that the endothelial glycocalyx is carbohydrate enriched layer on the endothelial cells in the blood vessels; broadly it may be considered to be a meshwork consisting of soluble molecules.[4] These molecules are derived from plasma and/or endothelium. Under normal conditions there exists a dynamic balance between the flowing blood and the soluble components of glycocalyx; this equilibrium affects the thickness and the composition of glycocalyx. Based on the nonquantitative studies on glycocalyx it is made of proteoglycans, glycosaminoglycan, and glycoproteins. Proteoglycans include syndecans and glypicans, other being mimecan, perlecan, and biglycan, these may diffuse in blood stream but reside in glycocalyx.[5,6] There are five glycosaminoglycans, namely, heparan sulfate, chondroitin sulfate, dermatan sulfate, keratan sulfate, and hyaluronan; these have varied functions.[7,8]

Although glycocalyx is a common feature of freely moving cells such as circulating blood cells and the composite cells present as lining cells in case of blood vessels, alimentary canal, urinary bladder, ureter, urethra etc., Another transmembrane protein called selectin-L has been located on the membrane of white blood cells; it is concerned with the identification of oligosaccharide which is located on the endothelial cells. In multicellular organisms, such oligosaccharides act as markers to recognize the specific type of cells for interactions.[9] Mia *et al*. mentioned that in case of rat fat pad under sheer stress for 5 h the components of glycocalyx are in communication with the cellular cytoskeleton linked. They found that the distorted dense peripheral actin bonds and vinculin gets drifted to periphery of the cells of rat fat pad when subjected to sheer stress for 5 h.[10] Glycocalyx may exhibit variation in thickness, adhesive ability and other surface properties and conformation in different types of cells. Weinbaum *et al*. reported the glycocalyx to be 500 nm in thickness toward luminar side; this layer exhibits mechanical and biochemical features such as morphological changes due to fluid sheer stress, degree of penetration of microvilli of leukocytes, ability to regains original shape and size after white blood cells pass through in the tightly fitting capillaries. The glycocalyx shows variations because of the colloid osmotic pressure and other rheological effects resulted due to movements of body fluid through very fine capillaries. Glycocalyx respond to sheer stress caused by the fluid and this impact is passed on to cytoskeleton related to endothelia; these responses include inflammation and intracellular signaling.[11]

Glycocalyx of endothelial cells is present on the luminar region of these cells exhibiting antithrombogenic features and brush-like appearance. Hyaluronic acid and heparin sulfate are present in glycocalyx and are responsible for overall negative charge and this charge of glycocalyx renders it highly hydrophilic in nature. Generally, a surface having hydrophilic nature is not likely to adsorp nonspecific proteins in comparison to the surfaces having hydrophobic surfaces but water binds strongly to negatively charged surface; this binding is so strong that bonded water cannot be replaced by proteins. The thickness of glycocalyx may change within the range from few nanometers to 3 μm; endothelium of blood vessels and changes in its thickness both are likely to affect the function of glycocalyx during thrombosis. Reitsma *et al*. suggested that the glycoprotein and proteoglycans form a network specifically on the luminar side of the cells.[4] Biochemically the components of glycocalyx such as proteoglycans, glycoproteins and glycolipids are arranged to give "hair-like" appearance; the carbohydrates are connected to proteins via covalent bonds, but additional glycoproteins and polysaccharides are bonded noncovalently. Part of these molecules is interlinked with the components of extracellular matrix. This results in the interlinking of extracellular matrix, glycocalyx and cell membrane.[12]

Functional Aspects of Glycocalyx

The functional aspects of glycocalyx are dedicated to maintain its healthy functional state; proteoglycans act as "backbone" and connect the glycocalyx and the cell membrane of endothelium.[4] The adhesive molecules on the cell membrane of endothelial cells participate in cell signaling involving cells from blood stream; of these, selectin, integrins, and immunoglobulin are prominent. Other functions include vascular permeability;[12,13] expressed that controlling factors for stress caused due to the colloids, fluids on the vascular endothelium;[14] to check the approach of molecules to endothelial cells and interactions between blood cells, platelets and wall of the vessel;[15] to adjust rheological fluctuations due to blood flow/body fluids;[16,17] to regulate the interaction between stimuli such as enzymes, cytokines, and reperfusion on adhesion molecules;[15,18] as mechanotransducer.[19] Glycans are the component of glycocalyx that covers the cellular surface and this aspect is very closely associated with cell biology and cellular technology. When the glycans get elevated there is enhanced cellular proliferation and cellular differentiation of the specific cell type; it has been observed that during transplantation, the glycans of glycocalyx stimulate and modulate the signaling molecules such as "fibroblast growth factor" and notch signaling molecule. These signaling molecules are concerned with cell division differentiation and cell death.[20] The glycocalyx under specific conditions facilitates cooperative binding and clustering of adhesion receptors.[21] Glycocalyx acts as a potential vascular barrier and its damage can lead to vascular pathology because it is susceptible to the sheer force exerted by blood flowing through the blood vessels; possibly this feature is responsible for the variations in the thickness of glycocalyx (0.01–1.0 μm).[22] Glycocalyx affects the functional ability of cell membrane

such as cellular adhesion, cell to cell recognition, cellular messaging/cell signaling. The negative charge of glycocalyx is due to glycans; this parameter acts as a regulatory factor for flow and mechanotransducer, interaction with leukocytes and regulation of coagulation.[23] Damage to glycocalyx influences the permeability of molecules having higher molecules through inter-endothelial pore (fenestrated zone), uncontrolled growth factors, cytokines, and increase in interstitial cell matrix.[23] Glycocalyx–a dynamic lining when gets deformed causes pro-atherosclerotic, ischemia, adhesion of blood cells specifically leukocytes, concentration of low-density lipids, pathological conditions such as hyperglycemia, mismanagement of intravenous fluid, and renal diseases.[24] There are some of the pathophysiological or clinical conditions such as hemorrhage shock, defective functional microcirculation, edema, inflammation, defective coagulation, disoriented and abnormal arterial pressure, all such and similar conditions cause derogative effects on glycocalyx. Adversely affected glycocalyx exhibits changed or deformed size, thickness, uncontrolled rolling of blood cell and adhesion.[25] The interactions between ligands present on the circulating tumor cells and the selectin-E adhesion molecules play an effective role in regulation of endothelial glycocalyx. The findings of their experimentation revealed that metastasizing cells secrete some of the molecules that bring about destruction of endothelial glycocalyx and the destructed sites are the sites of adhesion.[26]

Changes in Glycocalyx during Cancer Metastasis

The structural orientation of glycocalyx is very crucial as it bridges the cell membrane and extracellular matrix in any of the tissue not only in eukaryotes but also in prokaryotes. This aspect represents its structural and functional significance, in the recent past extracellular matrix has gained attention of the researchers[2] and the glycocalyx.[7] It is envisaged that both of these aspects of a tissue have their structural and functional impact under normal and also under clinical or pathogenic conditions. Under this presentation, an effort is being made to present an overall view of glycocalyx specifically with reference to cancer/metastasis.

The glycocalyx of circulating tumor cells and endothelial cells of the blood vessels are the probable aspects that regulate the spacing and the site of interactions between receptors and ligands. The glycocalyx of circulating tumor cells is also concerned with its binding with therapeutic binding within blood stream. The probable origin of cancer cells is primary tumor; the affected cells get detached, invade the ambient tissue and finally escape into the vascular system and circulate as circulatory tumor cells. These can comfortably enter and exit via postcapillary venules of the uninvaded tissue. These cells most likely exhibit the interactions related to adhesion at endodermal glycocalyx of the microvessels and undergo prolific cell division resulting in the formation of secondary tumor.[27] The process of interaction between circulating tumor cells and the glycocalyx of vulnerable endodermal of blood vessel can be compared with "seed and soil" hypothesis.[28] The metastasis of tumor cell is related to the involvement of homeostatic favoring factors which enhance the tumor growth, their survival, angiogenesis, and invasion.[28] The permeability of the cell membrane and the size of permeate are among the functional parameters for any biological system may it be homeostatic state or pathophysiological state. The circulating tumor cells have to move across the endodermal layer of vascular circulation. The size of circular tumor cells and the permeability of the endothelial layer of the vessels are of significance. Endothelial glycocalyx of blood vessels and microvessels acts as a permeability barrier and also provide a means of mechanotransduction effective forces and hydrodynamic sheer forces. In this respect, cytometric changes in the circulating tumor cells and the thickness of glycocalyx are the two functional factors that are likely to affect the process of migration through the wall of microvessels.[10] The thickness of glycocalyx, endothelium of blood vessels and microvessels and the size of the circulating cell undergo changes. Lazar *et al*. found the area of prostate cancer circulating cells was found to be within ~50–130 μm^2 and the area of cell of prostate cancer cell line ~150 ± 28 μm^2; in case of ovarian cancer the area of circulating tumor cells was 138 ± 45 μm^2 in comparison to the area of leukocytes 51.8 ± 8.3 μm^2; in case of breast cancer the area of circulating tumor cells was 135 ± 38.8 μm^2 in comparison to the area of leukocytes 48.0 ± 3.8 μm^2.[29] The thickness of endothelium is affected by the ability of circulating tumor cells to overexpress some of the building molecules of glycocalyx and these help in the progression of tumor resulting in enhanced angiogenesis, growth of the tumor and invasion; these are enhanced because of the ease of the interaction between the small size of receptors (around <100 nm) and the reduced thickness of glycocalyx of microvessels (less than or around 0.5 μm).[30] Under these conditions, the circulating tumor cells can easily move across the cell membrane or the wall of microvessels. The reduced thickness of the microvessels is vulnerable to circulating tumor cells and also it is prone to the adhesion of the circulating tumor cells.[30] The cytometric studies related to various cancer cells and circulatory tumor cells project a specific profile that can provide most probable fundamentals for nonevasive diagnosis, monitoring, and evaluation of cancer although these studies are very old because of the advancements in the technologies relation to methodologies have attracted the researchers to indulge in similar studies.[31]

Malignancy is correlated with the changes in the glycocalyx of the cells in the tissues. Its expression is dependent on the spatial orientation and functional aspects of transmembrane receptors.[32] The glycoproteins present in the glycocalyx are capable to elevate the degree of adherence of integrins, thus bringing them very close to each other; as a result of this cell-to-cell signaling gets activated or disturbed. This is accomplished by causing tensions in the membrane-bound integrins and during this process the contractibility of actomyosin is also affected.[32] Tumor circulating cells exhibit bulky/robust glycocalyx during malignancy and this modification facilitate the cell-surface receptors in mechanical mode. The bulky glycocalyx over all bring about (a) clustering of membrane-bound integrins by converging them and (b) change the state of integrins by elevating the tension on the

matrix bound integrins. The impact of these changes results in promoting adhesion causing activation of growth factors dependent on integrins, this further results in elevated signaling and enhances cellular growth and survival.[32] Glycocalyx appears to be more "robust" during metastasis; in tumors, there is a clustering of integrins, increased signaling of growth factors; this is accompanied by increased flow sheer stress due to activation of mechanotransduction within interstitial ambient environment of tumors.[33] Such activities in the nearby matrix of the tumor and/or metastasizing tissue elevate the release of metalloproteinase that are responsible for the enhanced cellular mobility and metastasis. In cancer cells, heparan sulfate and hyaluronic acid are elevated adding to the growth of the tumor and rate of metastasis.[33] When plasma colloid osmotic pressure declined below 16 mm Hg, the components of glycocalyx-like syndecans-1 and hyaluronic acid were shed, but chondroitin and heparan sulfates did not, thrombin production declined resulting in defective permeability and coagulation.[34] The glycocalyx undergoes shedding due to enzymes such as matrix metalloproteinases, reactive species of oxygen and nitrogen, and irregular or decreased shear stress. As a result, the barrier functions such as protection or holding of adhesive molecules, enzymes, signaling molecules mechanotransductive functions are adversely affected.[35] Glycocalyx encapsulates mammalian cells, and endodermal cells of blood vessels have potential to sense and act as a transducer for shear stress caused by flowing of blood and convert them into intracellular signals while tumor cells when exposed to body fluid (interstitial fluid) can influence the metastatic abilities. These abilities of "glycocalyx mediated transduction" of tumor cells appear to an important parameter to increase metastatic cell motility and initiate invasion.[36] When shear stress is extended for longer duration, it result in remodeling and make glycocalyx "robust." The glycocalyx components such as heparan sulfate and chondroitin sulfate glypican-1 and syndecans-1 get distributed uniformly spaced in the apical zone while caveolae1/caveolae and actin were concentrated at apical zone of the cells.[37] When tumor cell/cancer cells were treated with cATP (?) under the influence of vascular endothelial growth factor (produced by tumor cells), there is decline in the permeability and cell adhesion; the tumor cells were found to adhere at the cell junctions between endothelial cells but not in the cell bodies. There appears to be a possibility of strengthening the integrity of wall of the microvessels, which may lead to prevent metastasis of blood cells.[38] The increased apoptosis in the endothelial cells and shedding of glycocalyx of endothelial cells increases the permeability of low-density lipoprotein and facilitate plaque formation.[39] Human dermal microvascular endothelial cells lose its permeability, and it is in relation with sustained phosphorylation and/or activation of signal transducer along with activation of transcription and increased expression of mRNA. The knockdown of STAT3 gene by siRNA elevates the permeability of endothelium involving the tumor necrosis factor-α and Src family kinase.[40] Interaction between ligands located on the circulating tumor cells and the molecules of E-selectin that are present on the endothelial cells of brain can interfere in preventing the metastatic lung cancer cells; these cells are protectively covered by robust glycocalyx, vascular endothelial growth factor and tumor necrosis-α, which helped the adhesion of tumor cells to the endothelial cells of microvessels in brain.[41] Red blood cells get deformed due to shear stress/forces or deforming forces affecting rheological behavior of blood flow in blood vessels. If the deformity is beyond limits then there are chances of defective tissue perfusion, changes in cell shape, cell surface area, viscoelastic properties, cytosolic viscosity and membrane shear modulus.[42] Cell motility in all probabilities can be represented by cell stiffness, an important mechanical feature and it can be evaluated by atomic force microscopy involving quantitative parameter modeled Young Modulus; this concept is considered as biomarker for cell motility specifically for cancer cells.[43] Single cells exhibit intrinsic mechanical features that are related to cancer and senescence; these intrinsic features can be exploited for diagnosing early cancer cells. The elasticity and stiffness of cells vary in normal cells and in cancerous or metastatic cells because metastatic potential of cancer cells is inversely related to the respective cellular elastic features. The aging processes also be evaluated based on cellular stiffness because stiffness increases due to enhanced cytoskeletal fibers during the process of senescence.[44] Many diseases such as infections caused by viri (viruses) and related to immune responses and progression of metastatic tumor cells are significantly affected by "sialoside" - a synthetic sialic acid-containing carbohydrate.[45] Innate and adaptive immune destruction can be carried out by tumors; the sialosides on the cell surface can act as "backbone" of immune modulation. The therapeutic applications that are focused on sialosides associated with tumor may be an effective agent as anti-tumor-immunity. A selective desialylation of glycocalyx of tumor cells may have the potential to affect immune response by developing sialdase-conjugated antibody; sialated glycans can also be synthesized as glycocalyx and the same can be used to remodel the glycocalyx of cell surface.

Conclusion and Perspective

Glycocalyx covers the cellular surface and this aspect is closely associated with cell biology and cellular technology.[20] In spite of all the advancements in the direction of understanding, the mechanism of metastasis and pros and cons of oncology cancer still remains untamed lethal disorder. Tissue is one of the most complex entities of a biosystem and this complexity appears to be difficult to deal with. The integral components of tissues and their interaction with prometastatic biomolecules or any related agent is a challenge to meet and glycocalyx is no small matter. There are techniques such as radiations, chemotherapy etc., to deal and treat localized tumors and cancerous tissues but circulating tumor cells are potent enough to enhance the progress of the metastasis as these escape normal methodologies of treatments. The probable origin of cancer cells is primary tumor; these get detached and invade the ambient tissue and finally escape in the vascular system thereafter circulates as circulatory tumor cells. The cytometric studies related to various cancer cells and circulatory tumor cells project a specific profile that can provide most probable fundamentals for nonevasive diagnosis, monitoring and evaluation of cancer. During

metastasis the glycocalyx becomes "robust" and causes clustering of integrins, increased signaling of growth factors, this is accompanied by the increased flow sheer stress due to activation of mechanotransduction within interstitial ambient environment of tumors. The "robust" glycocalyx results in (a) clustering of membrane-bound integrins by converging them and (b) change the state of integrins by elevating the tension on the matrix bound integrins.[32] The overall impact is irregular cell proliferation, migration and signaling, all these factors promote mutagenesis. If adequate attention is paid to the functional, structural and molecular aspects of glycocalyx by exploiting the modern techniques and methodologies such as fluorescence microscopy, cytometric and rheological studies, and remodeling of glycocalyx of cell surface, some basic root cause may be envisaged that may provide some hope to deal with cancer at least to some extent.

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Conflicts of interest

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