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REVIEW

Electron microscopy approach for the visualization of the epithelial and endothelial glycocalyx

Visualisation du glycocalyx épithelial et endothélial par une approche en microscopie électronique

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Chemical contrast

Summary This study presents a methodological approach for the visualization of the glycocalyx by electron microscopy. The glycocalyx is a three dimensional network mainly composed of glycolipids, glycoproteins and proteoglycans associated with the plasma membrane. Since less than a decade, the epithelial and endothelial glycocalyx proved to play an important role in physiology and pathology, increasing its research interest especially in vascular functions. Therefore, visualization of the glycocalyx requires reliable techniques and its preservation remains challenging due to its fragile and dynamic organization, which is highly sensitive to the different process steps for electron microscopy sampling. In this study, chemical fixation was performed by perfusion as a good alternative to conventional fixation. Additional lanthanum nitrate in the fixative enhances staining of the glycocalyx in transmission electron microscopy bright field and improves its visualization by detecting the elastic scattered electrons, thus providing a chemical contrast.

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Contraste chimique

Résumé Cette étude présente un protocole expérimental pour la visualisation du glycocalyx en microscopie électronique. Le glycocalyx est un réseau tridimensionnel principalement composé de glycoprotéines, glycolipides et protéoglycanes associés à la membrane plasmique. Il suscite depuis moins d'une décennie d'un regain d'intérêt en raison de son rôle majeur dans la physiologie et pathologie vasculaire. Dès lors la visualisation du glycocalyx requiert des techniques adaptées car sa préservation reste difficile, dû à son organisation fragile qui reste particulièrement sensible aux différentes étapes de préparation pour la microscopie électronique. Dans cette étude, la fixation chimique par perfusion a été réalisée et reste une bonne alternative à la fixation conventionnelle par immersion. La supplémentation de la solution de fixation par le nitrate de lanthane permet de réhausser le contraste du glycocalyx en microscopie électronique transmission champ clair et d'utiliser la diffusion élastique des électrons pour son observation en contraste chimique (STEM-HAADF).

Introduction

The outer surface layer of the plasma membrane is covered by a complex gel like network named the glycocalyx mainly made up of oligosaccharides chains linked to lipid, intrinsic protein and absorbed peripheral proteins of the cellular membrane. Glycocalyx covers large amount of cellular type as suggested by Bennet [1] and was observed by electron microscopy for the first time by Luft [2]. Its appearance in fine structure varies from cell to cell as well as its composition in glycoproteins, glycolipids and proteoglycan. Those structural differences contribute to the wide biological functions of the glycocalyx based on the properties of its constitutive membrane glycoconjugates.

Intestinal glycocalyx

In the intestinal epithelium, the glycocalyx is imaged as a uniform layer of dense filamentous material, surrounded by the microvilli of the enterocytes and is rich in glycoproteins and glycolipids. Glycoproteins have oligosaccharides chains covalently bounded to the peptide backbone by N-linkage to the asparagine amino acid. The carbohydrate chains are generally branched and contain sugar residues as acetyl glucosamine, acetyl galactosamine, galactose, mannose, glucose and sialic acid (or neuraminidic acid). The terminal position of the sialic acid provides negative charge to the glycocalyx surface [3]. In addition to oligosaccharides polymers, specific hydrolytic enzymes (like disaccharidase or peptidase) and glycosyltransferases as well as protein carrier, are referred to reside inside the intestinal glycocalyx. Because of its vicinity to the extracellular medium, glycocalyx ensures firstly a protective role of the enterocytes membrane against proteolytic degradation [4]. Furthermore, the specific glycosyl pattern, exhibited at the outer surface membrane, is involved in cell recognition and adherence. As a consequence, glycocalyx may play a key role similarly to antigens, but also as a receptor for hormones, interferons, lectines, pathogenes and may contribute to a turnover process or a molecular trafficking.

Endothelium glycocalyx

For the past decade, several studies have described the endothelium glycocalyx, as an important factor in vascular physio- and pathology [5], giving rise to increase knowledge about its composition to have a better understanding of its structure and function relationships [6]. Heparan sulfate, chondroitin sulfate, dermatan sulfate, keratin sulfate and hyaluronic acid are identified as the five major glycosaminoglycans (GAG), where the heparan sulfate and chondroitin sulfate are the most abundant proteoglycans in the vascular endothelium glycocalyx. The glycoproteins, which form the endothelium glycocalyx, belong to the proteins' family of immunoglobulins, integrins and selectins. The last one is predicted to have an important role in the cell-cell interaction, especially between leucocytes and endothelial cells. Embedded in this glycosyled matrix, are found soluble plasmatic proteins and proteoglycans to preserve the integrity of the glycocalyx. In this organization, chains of hyaluronic acid link each other by hydrogen bond, to form strength and long hyaluronan-hyaluronan complex as illustrated in Fig. 1 and constitute a highly-hydrated gel which maintain an osmotic pressure to the surface of the matrix [7,8]. In addition to these properties of recognition site and cell interaction, endothelium glycocalyx is described to have an important action in the vascular permeability [9] by limiting the movement of molecule and water flow closed to the tight junctions [8,10] and, by the way, to preserve the homeostasis. Settled as a real vascular barrier, the most relevant involvement of endothelium glycocalyx is its role in the mechanotransduction regulation and the associated ''shear-stress'' effects [11]. Relationships between shear stress and morphological changes of endothelium are now well advanced restoring an interest for the investigations of the glycocalyx ultrastructure by electron microscopy [11—13]. Such approaches require reliable techniques to preserve the glycocalyx in a native state and to image it correctly in term of contrast. The appearance of the glycocalyx depends really of the procedure employed in the electron microscopy preparation. A dynamic but fragile structure, the glycocalyx is rapidly degraded when it is removed from its natural environment [14]. Furthermore, its glycosyled