Research Article

Insights in Blood Disorders

Morph Functional Features and Approaches to the Study of the Golgi Complex

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ABSTRACT

The Golgi complex is distinguished by great polymorphism and shape variability. The Golgi complex response to pathological disorders is predominantly as an intermediary component where it is involved in processing, sorting and secretion of materials via secretory granules, and in the formation of lysosomes. Initial response of the Golgi complex to any stress is an alteration or cessation of secretory activity. In the transformed cell, the Golgi complex is altered both morphologically and biochemically, suggesting a shift from a secretory to a membrane-generating mode of functioning. The study of the Golgi complex morph functional features will serve as a fundamental basis for distalization of the pathogenesis of many diseases.

Keywords

Golgi complex, Cell pathology, Research methods.

Structure and Functional Role of Golgi complex

Electron microscopic studies showed that the Golgi complex is formed by several components:

1. A system of flattened tanks, which are bounded by paired, smooth membranes (lamellae). Flattened cisterns usually lie in packs of 5-8, tightly adjacent to each other.
2. Small, rather dense microbubbles located at the ends of flattened cisterns.
3. Large vacuoles, limited by the same membranes as the cisterns. They are usually located in the middle part of a pack of flattened cisterns outside of them or between them [1,2].

There is evidence that the Golgi membranes, as well as the mitochondrial membranes, endoplasmic membranes and the cell membrane, have a three-layer structure and consist of a double phospholipid layer located on the surface of the protein layers. Flat cisterns form piles, which with the convex side face the nucleus (cis surface), and the concave side toward the cytolemma (Trans surface), Figure 1.

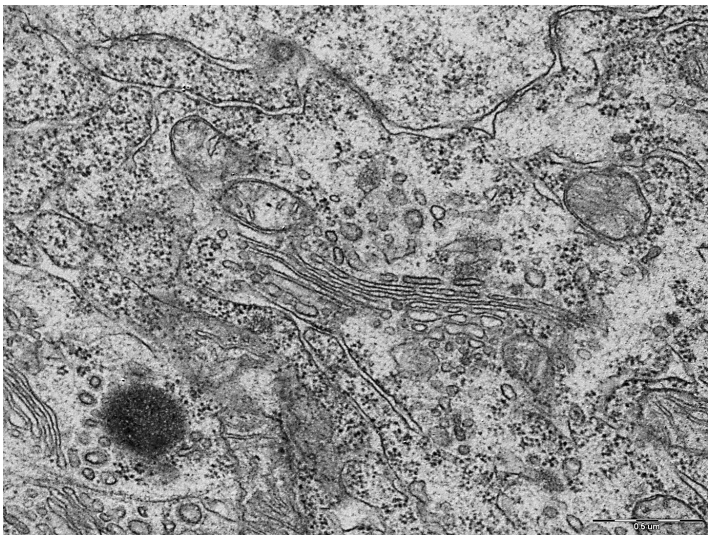

Figure 1: Golgi complex of neurons in the inner pyramidal layer of the rat parietal cortex. Magnification: x50000. Electron gram.

Between them are the cisterns of the medial part of the Golgi complex. Bubbles from the endoplasmic reticulum are poured

into the maturing cis-surface, and vacuoles are separated from the mature trans-surface, intended for exocytosis or the formation of endosomes and primary lysosomes [3,4].

The various components of the Golgi complex are interconnected and can arise from each other. Microbubbles are formed by lacing from the ends of the flattened tanks. Large vacuoles arise through the expansion of cisterns, and, flattening, can turn into a system of cytoplasmic membranes. According to other data, microbubbles are derivatives of "transitional" sections of the endoplasmic reticulum [5].

Arginine, small amounts of RNA, and alkaline phosphatase were detected in the Golgi complex. Histochemical, respiratory enzymes in the Golgi complex could not be detected and only a weakly positive reaction to lipoamine dehydrogenase and NADP-diaphoresis was revealed [6].

Biochemical analysis showed that the Golgi complex contains equal amounts of phospholipids and protein. The Golgi fraction contained a small amount of RNA and lipoamine dehydrogenase. The absence of specific biochemical features and the relative poverty of enzymes in the Golgi complex made it possible to believe that the function of this organoid is carried out not by enzymatic means [6,7].

It was found that the formation of secretions (enzymes) begins by synthetic processes in the ribosomes. The synthesized secretory material enters the intracisternal spaces of the granular endoplasmic reticulum, where the formation of primary prezimogen granules occurs. Through the smooth endoplasmic reticulum, the secretory material enters the Golgi complex, where the final formation and maturation of secretory granules takes place [8]. Concepts were developed about the principle of an intracellular "conveyor" in the production of enzymes. According to these ideas, in the cell “factory” that produces the secret, the Golgi complex serves as the last “packing shop” in which the secretory material is condensed and formed [9-13]. Considering the poverty of the Golgi complex of enzymes, one can imagine that this condensation of secretory granules occurs not by enzymatic means, but due to the osmotic removal of water [14,15].

The complex takes place:
- Phosphorylation and sulfation of protein, its partial cleavage (processing), the formation of complex complexes between proteins, carbohydrates and lipids synthesized in the endoplasmic reticulum.
- Accumulation and packing of secretions into membranes.
- Removal of the secret from the neuron.
- The formation of primary inactive lysosomes.

Thus, the Golgi complex is an organelle in which materials accumulate, condense and pack, which go to the construction of various intracellular inclusions. It serves as one of the last sections of the cellular "conveyor" that completes the formation of inclusions [1,2,9-12,16].

Role in Human Pathology

The Golgi complex response to pathological disorders is predominantly as an intermediary component where it is involved in processing, sorting and secretion of materials via secretory granules, and in the formation of lysosomes. Initial response of the Golgi complex to any stress is an alteration or cessation of secretory activity. In the transformed cell, the Golgi complex is altered both morphologically and biochemically, suggesting a shift from a secretory to a membrane-generating mode of functioning. However, since fewer or less well-developed Golgi complex are frequently found in transformed cells, analytical methods of membrane isolation developed for normal tissues may not always yield equivalent results when applied to tumors. Some lysosomal dysfunctions may result from under glycosylation of acid hydrolases by the Golgi complex [16].

Functional changes of the Golgi complex include changes in Golgi pH, aberrant Golgi glycosylation, and membrane trafficking. Golgi fragmentation has been found to often be an early causative event in the process of cell apoptosis. The nucleus signaling pathways of the Golgi stress response was identified in a previous study: The procaspase-2/golgin-160, TFE3, HSP47, and the CREB3-ARF4 pathways. If these pathways fail to repair overstimulation, the Golgi is completely disassembled, inducing cell apoptosis [11,12].

Apoptosis triggered by structural changes and functional disorder of the Golgi contributes to the pathogenesis of many diseases, such as neurodegenerative diseases, cardiovascular diseases, ischemic stroke, pulmonary arterial hypertension, infectious diseases, and cancer [9,10].

Neurodegenerative Disease

Structural and functional changes of the Golgi complex are associated with several neurodegenerative diseases, such as Huntington's disease, lateral sclerosis, Alzheimer's disease, Parkinson's disease, Creutzfeldt-Jacob disease and multiple system atrophy. Golgi fragmentation may alter neuronal physiology, and induce failures in transport to axons, dendrites, and synapses [9,16].

Finally, Golgi alteration may trigger a stress response and, as consequence, result in neuronal death. Furthermore, Golgi fragmentation in neurodegenerative disease alters protein trafficking and production, such as amyloid precursor protein in Alzheimer's disease, and sodium-dependent vitamin C transporter 2 in Huntington's disease. Alteration of the microtubule and microfilament stabilization may also be the cause.

In Alzheimer's disease and other tauopathies, tau-induced microtubule bundling may result in Golgi fragmentation. Furthermore, perturbations in Golgi pH are also responsible for Golgi fragmentation. The Purkinje cells from the Golgi pH regulator conditional knockout mice exhibited Golgi fragmentation, followed by axonal degeneration and neuronal loss Cancer [6,13].

Aberrant Golgi glycosylation regulates invasion of cancer cells, such as in prostate, breast and gastric cancer.

Golgi glycosylation is involved in basic molecular and cellular biology processes occurring in cancer, such as cell signaling transduction and communication, cancer cell dissociation and invasion, cell-matrix adhesion, cancer angiogenesis, immune regulation and metastasis. Similar to epithelial cadherin, a transmembrane glycoprotein is involved in epithelial cell-cell adhesion in tumors. The Golgi glycosylation of N-linked glycan on epithelial cadherin can affect the epithelial-mesenchymal transition, which is related to the formation of metastatic lesions [8,17].

This process is suggested to help cancer cells leave their original position during wound healing and other normal physiological processes, which is an essential mechanism for metastasis and diffusion of cancer cells.

The GOLPH3 complex is an important molecular component in the process of Golgi-driven tumor progression.

In addition to GOLPH3, the Golgi protein GM130 is important in Golgi glycosylation and protein membrane trafficking in cancer cells. Downregulation of GM130 induces autophagy, inhibits glycosylation, decreases angiogenesis, and suppresses tumorigenesis [10,13].

Structural changes and functional disorder of the Golgi complex have been identified in many cardiovascular diseases, such as heart failure, dilated cardiomyopathy, arrhythmia, and chronic Arial fibrillation [8,17].

For example, in dilated cardiomyopathy patients, morphological changes in Golgi vesicle are consistent with the secretion of natriuretic peptide as the rate of protein secretion affects the morphology and size of Golgi vesicles [15,5].

With cell pathology, the Golgi complex is deformed, its cisterns vacuole (Figures 2,3).

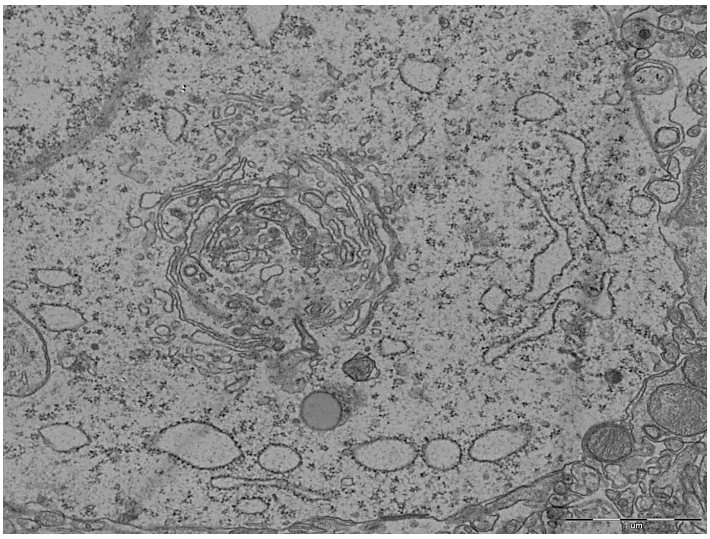

Figure 2: Pathological changes in the Golgi complex of neurons. Magnification: 40000. Electron gram.

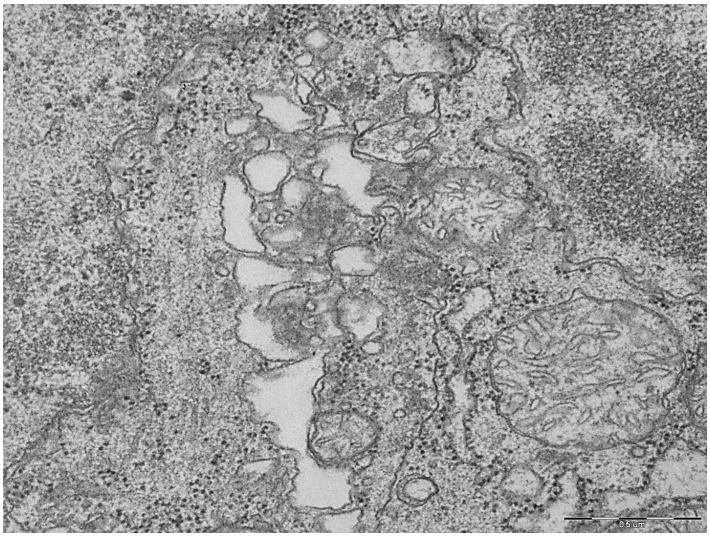

Figure 3: Pathological changes in the Golgi complex of neurons. Magnification: 40000. Electron gram.

Methods of Analysis of Structure of Golgi Complex

Golgi morphology after chemical fixation

Before the introduction of aldehyde fixation for electron microscopy in the early 1960s, metal salts as if osmium tetroxide (OsO4) generally fixed samples, whereas the permanganate fixation showed slight advantages on Golgi morphology. Using OsO4 fixation, the general architecture of Golgi complex—sometimes named 'dictyosome'— consisting of stacked membranes, surrounding vesicles was described at impressive clarity in sea urchin eggs, and exocrine cells of murine pancreas, with their important fixation details published elsewhere [18-21]. After the aldehyde fixation being established in the 1960s, electron microscopic research developed toward cytochemical labeling, which allowed understanding the functional morphology and cis–trans polarity by differential distribution of glycosylation enzymes along the Golgi stacks. Therefore, the main focus was on preserving enzyme activities after fixation and also on retaining antigenicity, taking slight compromises on the morphological appearance of the whole organelle into account [15,22].

Highlighting all aldehyde-fixation-based cytochemical methods like immunolocalization of antigens, correlative light and electron microscopy, and even three-dimensional protein localization would be beyond the scope of this article and was recently reviewed [23]. However, it should be noted that compromises on fixation procedures could lead to artifacts like very small membrane connections between adjacent cisternae. The misinterpretation of such results might induce wrong concepts on the functional morphology of Golgi complex. Nevertheless, our knowledge on the general Golgi morphology as described in current textbooks is based on impressive studies of the aldehyde-fixed organelle investigated in three dimensions [3].

Cryo-Fixation: Plunge Fixation

A major improvement on the fixation of biological specimen occurred with the introduction of cryo-fixation. Compared to

chemical aldehyde reactions, which crosslink biological material in a timescale of seconds to minutes, the velocity of cryo-fixation is far superior, since all biochemical, physiological, and dynamic processes are arrested during 10–20ms in their actual state by a massive temperature drop. The viscosity increases dramatically and the sample transforms into a 'glass' – meaning that it is completely embedded in vitreous or sometimes called amorphous ice. The vitreous specimens remain fully hydrated and are still liquid by physical definition, but share properties of solid matter as they are in fact in a very high viscosity state [24]. However, the process of verification is not completely understood yet. Constantly kept below the devitrification temperature of −140°C (for pure water), the 'glassy' sample will not flow in the timescale of a realistic experiment, even viewed at electron microscopic magnifications. To ensure the transition of the sample to a 'glassy state' and avoid any segregation of molecules by ice crystal growth, a cooling velocity up to 100,000 °C/s is intended. Thin samples such as purified macromolecules deposited on an electron microscopy grid, very small cells like bacteria or flat parts of eukaryotic cell periphery can be completely vitrified by simply plunge freezing them into an adequate cryogen [23,24].

The main advantage of studies performed on vitrified specimens is the preservation of their inherent native densities, revealing the natural arrangements of biological structures [14]. This means, we 'see' and image directly the membranes, ribosomes, fibers, protein complexes, and larger molecules by a direct interaction between those biological structures and the electron beam, not by a secondary detection of an artificial heavy metal impregnation. Furthermore, aggregation of biological structures—a phenomenon usually attributed to the dehydration process during resin embedding – is dramatically reduced [18,20].

Among the various approaches developed for cryo-fixation, plunge freezing is one of the earliest and was already used for the first electron microscopic characterization of the verification of pure water. For samples like larger cells or tissues, where the Golgi field is located in the perinuclear region, this technique is not suitable, as the freezing speed decreases rapidly from sample surface deeper into the bulk, inducing ice crystal growth. However, for small prokaryotic cells, plunge freezing is a surpassing fixation procedure leading to well-vitrified samples. Hence, the general view of bacteria, formerly seen as a 'bag of enzymes' was revolutionized by cryo-electron tomography of plunge-frozen samples, and today's understanding of cellular substructures like complex bacterial cytoskeleton and the architecture of various large macromolecular complexes emerged [21].

One of the smallest known eukaryotic cells is Ostreococcis Tauris, a unicellular green alga containing a single mitochondrion, one chloroplast, and one Golgi complex [16]. It is the only eukaryotic cell so far, which was effectively imaged in its entity by cryo-electron microscopy and tomography. Being able to plunge freeze and image directly the cells by electron microscopy, many steps of sample preparation are avoided, like post fixation, dehydration, embedding, sectioning, and staining, getting around all their potentially related artifacts. In high-quality cryo-tomograms, single Golgi stacks per cell have been identified, consisting of five cisternae without any luminal contacts and a low number of peri-Golgi vesicles [3].

Golgi Complex in Vitreous Sections

The application of CEMOVIS enables to resolve the details of Golgi complex formerly hidden in plastic-section electron microscopy. The general morphology of the organelle with 4–5 separate cisternae surrounded by peri-Golgi vesicles shows no structural differences between freeze-substituted versus vitreous material of the same cells [19]. At low-to-medium electron microscopic magnification (1,000x–20,000x), well-contrasted plastic sections give even substantial advantages compared to vitreous sections. At higher magnification, known structures as if vitrified clathrin coats give gentle contrast in their cellular environment, compared to purified samples in cryo-electron microscopy [17]. The COP coats of peri-Golgi vesicles appear as homogeneous or 'spiky' subtypes, whereas it remains unclear, if 'spiky coats' and 'homogeneous coats' represent distinct subtypes differing in genesis and protein composition, or if they are COP coats at different stages of coating/uncoating. However, their presence in systematically and evolutionary highly separated organisms such as humans and—for Golgi research very interesting—trypanosomes (Kinetoplastida) shows their ubiquity in eukaryotic cells. At least, a differentiation into COPIa and COPIb vesicles by size and content density—as recently introduced for freeze-substituted plant and algal cells can be neglected for mammalian and trypanosome Golgi vesicles, since interior densities and sizes of the 'spiky' and homogeneous coated vesicles are not distinguishable. Strong evidence for functionally different COPI vesicles comes from biochemical and recent immunoelectron microscopic data. However, it remains unclear, how slight composition differences would imply such structural diversification as observed by cryo-electron microscopy [18,21,24].

A new and unexpected finding at vitreous Golgi complex are protein complexes up to 6 nm in size and attached to cisternal membranes, as there is nothing comparable described in resin-embedded samples observed by electron microscopy, irrespective of freeze or chemical fixation and dehydration procedures. Their electron microscopic contrast is mainly phase contrast due to the wave function of electrons and related to the atomic potential distribution within biological molecules. After metal salt impregnation and dehydration, this phase contrast is concealed and overlapped by the amplitude contrast of stained material, detected through the particle behavior of electrons, whereas their phase contrast falls beyond detection. Some small pleomorphic complexes are attached to the luminal side of cisternal membranes, while others are localized between adjacent cisternae and could have stabilizing functions, but only a profound structural analysis and comparison to known Golgi-localized proteins will clarify their composition and function [5,22,23,25].

By cryo-electron microscopy, Golgi saccules have been shown 30–60 min after induction of procollagen secretion. This verified that such saccules exist also in vitreous ice-embedded samples and are not a sign of luminal swellings caused by local osmotic effects during inappropriate fixation or dehydration. Furthermore, during massive cargo transport, a luminal connection between cisternae was detected by cryo-electron tomography in vitrified Golgi complex. This was an interesting finding concerning the recent discussion about tubular continuities between different Golgi cisternae. Some of them have been found in nocodazole-treated cells undergoing a cargo wave of viral proteins, which was released after a low temperature-induced traffic block—of course a non-physiological experimental setup. In contrast to the first description of such tubules occurring during glucose-stimulated insulin secretion, with connections bypassing interceding cisternae in freeze-substituted samples, the cryo-electron microscopic data showed only one luminal continuity very central in a cisterna at a place of potential branching of the Golgi stack [19,24,25]. After revisiting the original cryo-tomograms, it became clear that much more and larger Golgi areas have to be analyzed to get conclusive evidence. Unfortunately, further and unambiguous luminal connections could not be traced after analyzing all cryo-tomograms of these experiments. In contrast, the cisternae seemed to be well separated from each other. However, the unique cisternal continuity detected in this cryo-tomogram was also very different from other luminal continuities between adjacent cisternae as described during synchronized viral cargo waves, as these were located at the outermost rim of medial cisternae, but not in the center of the stack [3,4].

Thus, the Golgi complex performs many important functions in the cell and the study of its morph functional features will serve as a fundamental basis for deatlization of the pathogenesis of many diseases.

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