Pioneering Organelle Structural Biology: Golgi Apparatus Dysfunction in Parkinson's Disease, Neurodevelopmental Disorders, and Cancer

Daniel Joseph Gómez-Santos

Department of Biological Sciences, College of Science, California State University, East Bay, 25800 Carlos Bee Blvd, Hayward, CA 94542, USA; daniel.gomez@csueastbay.edu
daniel.gomez@csueastbay.edu; Tel.: +1 (925) 315-7142

Abstract: The Golgi complex (GC) dysfunctions in Parkinson's Disease (PD), neurodevelopmental disorders (NDDs), cancer, and organelle structural biology (OSB) can provide insights into therapeutic targets, gene therapy, and drug design. Primary defects and fragmentation within the GC are implicated in a wide range of neurodegenerative diseases. GC defects typically result in mislocation of proteins, accumulation of undegraded proteins, and impaired glycosylation of proteins. Inhibition of vesicular trafficking by α-synuclein (aSyn) may affect the dopamine-producing neurons and neuromodulators. GC regulates apoptosis during pathological mechanisms of neurological diseases and could provide new avenues in treatments through translation research. PD patients bearing the hereditary E46K disease mutation manifest the clinical picture of parkinsonism. How do we provide high resolution nanoimages of the GC during disease to capture dysfunction? Could we visualize the aSyn traffic jam between vesicles in the organelles ER and GC? OSB is emerging as a field as more technology advances and is more accessible. Structural studies of the GC will advance the field of neurological disease forward with an in depth understanding of dysfunction, fragmentation, and defects. Discoveries of the GC in PD, NDDs, and cancer would break new ground and provide translational medicine data of these diseases. Future research could be visualizing high angle annular dark field-STEM (HAADF-STEM) tomograms, cryogenic electron tomography (cryo-ET), multiplex correlative light and electron microscopy (cryo-CLEM), nanobody-assisted tissue immunostaining for volumetric EM (NATIVE) and using soft X-ray tomography (SXT) and computational reconstruction of the GC.

Keywords: structural biology; organelles; Golgi Complex (GC); Parkinson's disease (PD); cryo-ET; alpha-synuclein; neurodegenerative diseases; soft X-ray tomography (SXT); cancer; NDDs

1. Introduction

The emerging field of organelle structural biology is being pioneered and will see its impact in the coming years. There has been much progress in structural molecular biology that has laid the foundation for the organelle to be investigated. Even after a century of studying the Golgi complex (GC), it remains a fascinating organelle for cell biologists. This highly dynamic organelle is considered the "heart" of intracellular transportation. In cryo-EM, new data and improvements in data processing will become available in the next coming years. There won't be much complicated data processing and we will also most likely learn a lot. The rationale of OSB is that organelles are making changes within the cell that involve multiple macromolecules. Cryogenic electron microscopy and tomography (cryo-EM and cryo-ET) are the ideal techniques to investigate organelles such as the Golgi apparatus because it allows the organelle to behave in its native environment. The purpose of communicating this work is to consolidate recent and previous research in the Golgi apparatus and its role in neurorestoration and how neurotoxins can interfere and with communication with the endoplasmic reticulum (ER) and the Golgi complex (GC).

The Golgi complex has attracted the attention of cell biologists due to its "fashionable" morphology and central position with the secretory system of the cell. There is also protein sorting, processing, and multiple functions in the three-dimensional architecture. The GC is a potent microtubule (MT)-organizing organelle, where MT stabilization requires additional cytosolic factor(s) and that MT nucleation is directly involved in Golgi-bound γ-tubulin [1]. The principal structural component of the GC is the stack of cisternae; it is a highly ordered and polarized structure [2]. There are also clathrin coated vesicles on the trans-Golgi complex that form and are then loaded onto microtubules to be secreted outside the cell, loaded onto the cell membrane, or have some other destination within the cell. The focus will be on the advances in organelle structural biology, the structure and function of the GC in PD, NNDs, and cancer. The significance of the work will provide physicians, physician-scientists, and researchers with novel studies that have investigated the GC dysfunction in disease and how to target it with therapeutics. Structural biology has revolutionized medicine and will continue to be the standard for providing precision and personalized medicine. The current state of the Golgi complex in the PD research field is being led by great researchers around the world. All key publications are cited and included in this communication. There weren't any controversial or diverging hypotheses, aside from the reality of the GC when it was discovered by Camillio Golgi in 1898, when he reported a new cellular constituent (the apparato reicolare interno). No other controversies were found during the investigation but that doesn't rule out the possibility in the future. With the current state of technology, we will only be more informed of the structure of the organelle and its condition during disease. The main aim of the work is to reveal the organelle structural biology of the Golgi apparatus in dysfunction and disease. Newly synthesized proteins cross the ER membrane from the cytosol to enter the secretory pathway. These proteins are successively modified as they pass through a series of compartments from the ER to the GC and from the GC to the cell surface and elsewhere. There is a delicate balance between forward and backward (retrieval) transport pathways. The pathway from the ER to the cell surface consists of many sorting steps to continually select membrane and soluble luminal proteins for packaging and transport. The GC is a major site of carbohydrate synthesis, as well as a sorting and dispatching station for products delivered to it from the ER. The cell makes many polysaccharides in the GC such as glycosaminoglycans of the extracellular matrix. The GC can also build and attach oligosaccharide chains to many proteins and lipids that the ER sends to it. The oligosaccharides could serve as tags to direct specific proteins carrying them into vesicles that are then transported to endosomes for delivery to lysosomes. Most of the time they are targeted to transport vesicles going to the cell surface. When it comes to the identity of an organelle the Rab proteins can create or change this. Rab proteins function on organelle membranes; specifically, Rab GEF at the organelle catalyzes Rab protein activation and insertion at the membrane surface. There are effector proteins recruited by an activated Rab protein that help give the organelle its identity by directly controlling incoming and outgoing transport vesicles. The GC is a highly dynamic cellular organelle that functions to process and sort protein and lipids during trafficking from ER to different destinations in the cell [2]. The homeostasis of cellular proteins and lipids is affected by alteration in the structure and function of the GC. There is an increase in evidence that suggests structural and functional changes are involved in PD [3]. The Golgi begins to fragment and is dependent on Rab and SNARE. Alpha-synuclein (aSyn) is a key protein connected in PD pathology. PD pathology is characterized by the loss of dopaminergic (DA) neuronal cells in the substantia nigra pars compacta (SNpc) and abnormal accumulation and aggregation of aSyn in the form of Lewy bodies and Lewy neurites. This aggregation of aSyn is associated with dysfunctionality and degradation of neurons in PD. The striatal dopamine depletion causes dysfunction of basal ganglia circuitry [4] [5]. Progressive neurodegeneration disease pathological mechanisms involve neurotoxic protein misfolding, oxidative stress, and proteasomal impairment. Toxic misfolding in protein assemblies present mechanics links in neurodegeneration. Studies on autopsied substantia nigra of PD patients and experimental models propose gliosis as

a trigger for neuronal loss. PD has definitive motor dysfunction, yet non-motor symptoms exist like compromised olfaction, constipation, sleep disorders and various neuropsychiatric manifestations precedes the motor symptoms by several years [6]. Like viral-induced neurodegeneration, PD is characterized by T-cell infiltration, microgliosis, and astrogliosis [7, 8]. Chronic neuroinflammation is known to precede neuronal dysfunction during the asymptomatic stage. Overall, GC dysfunction contributes to PD (by aSyn and DA). The Golgipathies in early secretory pathway show defects and manifest NDDs with brain abnormalities. The Golgi in the nervous system if altered during the neurodevelopmental process from neurogenesis to neuronal migration as well as secretory function is crucial for maturation of postmitotic neurons and myelination. As these young neurons are migrating directionally to target regions there are heavy demands of synaptogenesis, synaptic and neurosecretory activity. This review discusses a myriad of NDDs such as Cohen syndrome, intellectual disability, mental retardation, deafness, postnatal-onset microcephaly, peripheral neuropathy, progressive childhood encephalopathy and incorporates different genes and proteins involved in the each NDD. Finally, the GC is ground zero for cancer and Golgi fragmentation is evidence of that during oncology. There are entire schematics for Golgi proteins and ligands and signaling molecules that contribute to carcinogenesis. Golgi fragmentation opens the doors for cascades of fatal pathways which may facilitate cancer progression and metastasis [9]. OSB satisfies this need for nanoimages through a myriad of OSB techniques (STX, CLEM, NATIVE, cryo-ET, cryo-EM, and HADDF-STEM). The implications of these future studies would serve all stated diseases (PD, NDDs, and cancer). Once a holistic understanding of the Golgi organelle is obtained by OSB we can begin to discuss therapeutic interventions.

2. Organelle Structural Biology

2.1. Golgi Complex

The GC is a highly dynamic cellular organelle that functions in sorting and processing of proteins and lipids during trafficking from the ER to various destinations in the cell [2]. The homeostasis of cellular protein and lipids are central to the function and structure of the GC. The GC consists of dozens of flattened, parallel, interconnected cisternae, and the pile of Golgi cisternae is referred to as the Golgi stack. These stacks can be divided into three functional compartments: cis-, medial-, and trans-Golgi cisternae. These stacks are connected to the cis- and trans-Golgi networks (CGN and TGN). Specifically, the first cis-Golgi cisterna is connected to the CGN and is involved in ER-Golgi trafficking. The TGN is associated with sorting of cargo to their destination. The GC is the central hub of the secretory pathway [10]. As the GC is a dynamic organelle whose correct assembly is critical for cell homeostasis, perturbation of the GC structure is associated with numerous disorders from neurodegeneration to cancer. There are functional links between proteostasis control and Golgi architecture that could be crucial in various secretion-related pathologies [11].
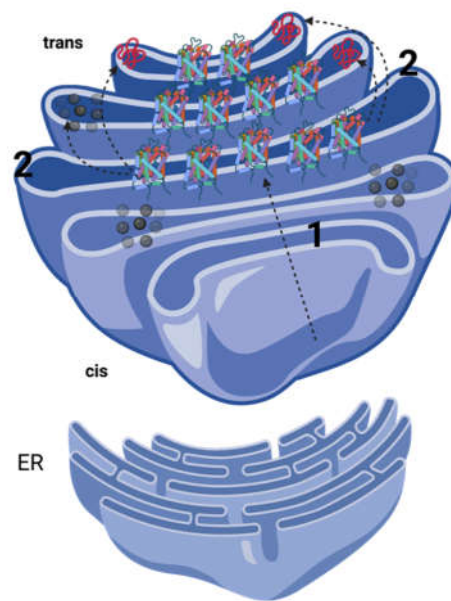
Figure 1. Traffic through the Golgi depicting intracisternal structures. Made with Biorender.

2.2. Structural Techniques

The greatest advantage of cryo soft X-ray tomography (cryo-SXT) is the ability to image unstained, whole frozen cells up to 10-um thick and resolution down to 30 nm [12] in a volume of a few microns [13]. When one studies the overall organelle organization or structural changes it's important to consider the context of the whole cell. It has been said that cryo-SXT can visualize the dance of the organelles. Using visible fluorescence microscopy (VLFM) or TEM could complement cryo-SXT and correlative workflows of which to put first are imperative. Using cryo-VLFM and cryo-SXT (cryo-CLXM) can describe specific organelles like autophagosomes identified [14]. High angle annular dark field (HAADF) is a scanning transmission electron microscope (STEM) technique (HAADF-STEM) that produces an annular dark field image formed at a very high angle with incoherently scattered electron as opposed to Bragg scattered electrons. HAADF-STEM tomograms give five times better contrast and signal-to-noise ratio that Bright Field TEM; template matching showed that 1.3 times more information could be extracted from HAADF-STEM tomograms than from Bright Field-TEM tomograms [15]. Cryogenic electron microscopy (cryo-EM) is a surfacing technique in structural biology and can resolve structural of macromolecules at resolution of 1.22Å for spherical molecules like apoferritin. These are outliers and most structures are around 2.5-3.5Å. It is true that anything above 3.5Å can be computed through structural bioinformatic methods (AlphaFold2 etc.). While cryo-EM must take another situation that is different than macromolecular crystallography (MX) like putting cells or sample into a vacuum which most living systems do not survive. When working with pure proteins this is more realistic. They are then bombarded by electrons to reveal "atomic resolution." It remains to be seen if cryo-EM can provide reliable structural information for subcellular structures such as organelles like the Golgi apparatus. There is a cohort at Scripps Research Institute, Grotjahn et al., that has worked out the surface morphometrics to quantify organellar membrane ultrastructure of the mitochondria. They revealed the membrane curvature and spacing through triangle mesh surfaces that represent the connectivity and implicit geometry of the membrane itself [16]. Cryo-ET is an optimal technique to study the GC in detail and in its native environment within a tissue architecture. We are getting closer to single cell and subcellular structures within tissue sections that cryo-ET could be the most modern optical imaging technique to reveal the organelle structure *in situ.* One could imagine screening a plethora of diseases from biopsies and necropsy tissue to elucidate pathology and etiologies. With automated annotation, neural nets, programs like

EMAN2, we can meet diagnostics with a high-throughput plan. A new quality step in Golgi research started with the development of correlative light-electron microscopy (CLEM) which is an innovative method that allows visualization of the fate of the same organelle of interest at any moment of its life span by first video imaging in live cells then immunoelectron microscopy and 3D reconstruction. One must consider how technically demanding this technique in nature; although, it proves to be very efficient and fascinating tool for studying Golgi structure and function [17]. One convincing technique published in 2018 by Lichtman et al. designs NATIVE that enables tissue-scale multiplex CLEM; this is an approach that preserves ultrastructure while showing locations of multiple molecular moieties, even deep within tissues [18]. NATIVE also enables correlative 3D volumetric reconstruction and using computer-assisted manual segmentation approach (VAST), one can segment both a nanobody-stained astrocyte and microglial cell. The next evolution would be to reveal subcellular structures like organelles. As we attempt to resolve the glycoproteome which is known to occur with synapse formation, there are N-glycosylation alterations to consider that result from the growth and proliferation of Golgi satellites scattered throughout the dendrite [19]. Being able to do these techniques in a specific sequence would be ideal to illuminate Golgi dysfunction in disease. A potential workflow of several cohorts could be cryo-SXT, VLFM, cryo-CLXM, HAADF-STEM, cryo-EM, cryo-ET, NATIVE, multiplex-CLEM, and VAST.

3. Golgi Apparatus Dysfunction in Diseases (PD, NDDs, and Cancer)

3.1. Golgi Apparatus Dysfunction

GC dysfunction is caused by primary defects within the Golgi or pharmacological and oxidative stress which has been implicated in a wide range of neurodegenerative diseases. The GC plays a crucial role in angiogenesis, neurogenesis, and synaptogenesis thus it promotes neurological recovery. It is now understood that it has a direct role in neurorestoration. GC fragmentation is known to be associated with mitosis and apoptosis. In many neurodegenerative diseases, there is a common feature of fragmentation of the Golgi ribbon. It is believed that there is an imbalance of ER-Golgi transport and/or cytoskeleton alterations. One group cultured the neuronal PC12 cell line with hydroxydopamine as cellular models of PD; they found that Golgi fragmentation precedes and could trigger the aggregation of aSyn and the formation of inclusions [3]. There were also anterograde and retrograde transport between the ER-Golgi complex and damage to cytoskeleton. Correlations between fragmentation and alterations in levels of Rab1, 2, and 9 and the SNARE protein syntaxin 5 were directly related. If these proteins are overexpressed the Golgi morphology is rescued. To understand the cytopathology of PD, it will be important to regulate the homeostasis of Rab and SNARE proteins. When aSyn aggregates and is misfolded, it is devastating for this neurodegenerative disorder. aSyn can block ER-Golgi traffic and Rab1 can rescue neuronal loss in PD models [20]. The aSyn accumulation does cause ER stress and impairs degradation of selective endoplasmic reticulum associated degradation (ERAD) substrates. This inhibition of ER-Golgi trafficking is a crucial aspect of aSyn-induced toxicity [20]. The severe blockage in vesicular trafficking in early secretory was shown by pulse-chase immunoprecipitation and compared to controls. Trafficking of carboxypeptidase (CPY) and alkaline phosphatase (ALP) was monitored in cells expressing aSyn-WT and aSyn-A53T. In the immunoblot, there is no clear indication that the p2 (Golgi form) has aSyn as there are only faint signals in all time frames. Looking at time of aSyn induction versus % of CPY remaining in the ER, aSyn-A53T was at 80% CPY remaining in ER after 4 hours, and around 60% in aSyn-WT after 4 hours. When adding galactose-inducible GYP8 and YPT1 or SLY1-20 there were similar results with all signals in p1 ER and m Vacuole. This demonstrates that aSyn induced cytotoxicity and vesicular trafficking defects are modified by ER-Golgi trafficking components. Lower levels of dopamine (DA) are characteristic of the PD brain and efforts of L-DOPA have been one of the most successful treatments in PD. DA is synthesized in the cytosol and quickly pumped by vesicular

monoamine transporter 2 (VMAT2) transporter into synaptic vesicles. If there are any defects in the early secretory pathway, this could cause a shortage of synaptic vesicles and there would be a reduction in VMAT2 delivery to the synapse. Thus, inhibition of vesicular trafficking by aSyn may affect the dopamine-producing neurons and neuromodulators. Another aspect of Golgi complex dysfunction is Golgi-related apoptosis which is associated with PD. The Golgi apparatus has possible mechanisms where GC regulates apoptosis. Our understanding of GC and apoptosis has been broadened when elucidating pathological mechanisms of neurological diseases and could provide new avenues in treatments through translational research. GC is a cellular sensor, adapting and changing its structure depending on the physiological state of the cell. There is a hypothesis that states the Golgi contributes to the polarization of membranes and if this a membrane of a neuron losing its polarity can affect the performance and overall survivability of the neuron.

3.2. aSyn Neuropathogenesis in Parkinson's Disease, Pathogenic Fibril Structure

Synucleins such as aSyn have fibrillar polymorphs that contribute differently to synucleionpathies. aSyn is 140 amino acids in length, and the SCNA gene is located at 4q23. More than half of each protein is taken up by six or seven imperfect 11-amino-acid repeats with the consensus sequence KTKEGV. Positively charged regions followed by hydrophobic regions and negatively charged regions in the carboxy-terminal region. By cryo-electron microscopy helical reconstruction was performed to determine the structures of the two predominant species, a rod and twister, both at 3.7Å resolution [21]. These atomic models show the rod and twister species both have a kernel structure of a bent β-arch but differ in their inter-protofilament interfaces. There are hereditary mutations in aSyn linked to these conditions. The aSyn fibrils containing the H50Q hereditary mutation results in new polymorphs such as narrow and wide fibrils. The mutations accommodate faster aggregation kinetics, higher seeding capacity in biosensor cells and greater cytotoxicity [22]. Hereditary mutations in aSyn from patients bearing the E46K disease mutation manifest the clinical picture of parkinsonism and create more pathogenic fibrils *in vitro*. The structure of the symmetric double protofilament structure was solved at 2.5Å. This could be contributing to a misfolding pathway as it unlocks a more stable and pathogenic fibril structure. In rats, aSyn is most abundant in telencephalon and diencephalon with lower levels in caudal regions. Recent studies have shown that it is a homotetramer with predominately α-helical conformation. Experimental studies have shown that aSyn binds to lipid membranes [23]. Lipid membranes containing acidic phospholipids have monomeric binding. This conformation is taken up by amino acids 1-98, with residues 99-140 being unstructured [24]. There are several missense mutations (A30P, E46K, A53T) that cause PD and dementia with Lewy bodies (DLB). Missense mutations in SNCA or increase in gene dosage (duplication or triplication) of the chromosomal region containing SCNA cause autosomal dominant inherited forms of PD and DLB. Overexpression of WT aSyn has been identified as a cause of PD and DLB [25]. Amino acid in position 53 was shown to be different in rodents (mice, rats) versus humans; in rodents it is T53 and in humans it is A53. This suggests that it is not the threonine that is pathogenic but instead there is a difference in conformation of mutation protein that is attributed to human, but not rodent aSyn. Lewy body filaments are made of aSyn. The GC has a conserved mediator of Ca^{2+}/Mn^{2+} ATPase, PMR1, residing within it; it can regulate aSyn-induced Ca^{2+} imbalance and cell apoptosis [26].

3.3. Golgipathies: Early Secretory Pathway Defects and NDDs with Brain Abnormalities

Taking into account the role of the GC in the nervous system there is an emerging concept of altered neurodevelopmental processes, from neurogenesis to neuronal migration and the secretory function that is crucial for maturation of postmitotic neurons and myelination [27]. Neurons and glia presumably make use of the GC through coordinated processes involved in progenitor cell organization, orientation, and mitosis.

The directional migration of young neurons to their target regions contribute to the heavy demands of synaptogenesis and synaptic and neurosecretory activity. There are Golgi outposts in dendrites which is a prime example of how the GC adapts to a highly compartmentalized nature of the nervous system and trafficking needs [27]. In Cohen syndrome (COH), there is a GC protein VPS13B which causes neurological signs such as visual impairment, and a short and thick corpus callosum. Mental retardation and deafness have the GC protein AP1S1 which can manifest as an intellectual disability (ID), postnatal-onset microcephaly (POM), and peripheral neuropathy. Progressive childhood encephalopathy (PEBAS) has the TRAPPC12 GC protein which can cause major neurological signs like POM, truncal hypotonia with appendicular spasticity, visual impairment, partial agenesis of the corpus callosum. Diagnosis by next-generation sequencing (NGS) of NDDs have variable and non-specific clinical findings. Whole genome sequencing (WGS) on members of an Ashkenazi Jewish pedigree have identified underlying genetic etiology of global developmental delay/intellectual disability in three affected siblings [28]. This study filled the gap in knowledge of TRAPP architecture with TRAPPC2L interacting with TRAPPC6a which could position it as a putative adaptor for other TRAPP subunits. The TRAPPC2L A2G variant is indeed pathogenic. Another GC protein family that transports copper P-type ATPases, ATP7A and B that are trafficked by AP1S1 which causes MEDNIK syndrome that the trans-Golgi and endosomes match cargo molecules including neurotransmitters to their carriers. The symptoms for MEDNIK include POM and moderate-to-severe ID and deafness [29]. ATP7A and B are ion pumps located in the trans-Golgi network (TGN) and translocate copper across intracellular membranes into the secretory pathway into copper-dependent enzymes. The childhood onset or dystonia and neurodegeneration of either childhood- or late-onset is caused by ATP7B. The stages of the central nervous system development start in the first trimester of pregnancy where there is neurogenesis and neuronal migration. The Golgi apparatus functions begin with the maintenance of Golgi apparatus structure, integrity and position, biosynthesis and modification of lipids and proteins, cellular polarity, microtubule organization, and cell cycle regulation. In the third trimester and birth, there is gliogenesis, neuritogenesis, and apoptosis. A little after birth, there is myelination, astrocytic expansion, synaptogenesis, and synaptic stabilization. All these processes continue for the first three years of life and adolescence. The GC has an essential role in the formation, growth, and maturation of the brain and nervous system.

3.4. Golgi Complex: Ground Zero for Cancer

The GC is ground zero for promoting cancer hallmarks and tumor progression. Golgi fragmentation was confirmed in cancer by electron microscopy almost sixty years ago yet only recently have we begun to understand the significance of Golgi fragmentation in the biology of tumors [9]. As evidence grows on the connection of the Golgi with cellular events related to cancer initiation and progression, there are now mechanisms of regulation of cell survival/death, proliferation, motility, metabolism, and immune evasion. An organelle fact is that Golgi-associated processes can impact cancer cell phenotype and consequently cancer progression. This does imply that there are potential anti-cancer progression pharmacological targets. We would have to consider the impact of the cell membrane protein and lipid repertoire and secretome during mechanisms of dysregulation of the GC [30] [31] [32]. Cell proliferation, cell death, extracellular environment, metabolic status, and cell invasion can all contribute to the cancer cell phenotype and cancer progression. While the mitochondria metabolism plays a major role in increasing ATP and ROS the Golgi assists in Ca^{2+} signaling and H^+ flows, controlling immune modulation and the glycocalyx, and relevant for cancer cell phenotype control. There is still more to learn about the Golgi Ca^{2+} homeostasis and H^+ flows. The sodium-hydrogen exchanger 7 (NHE7) [33] and TMEM165 [34] are involved with these Golgi-resident channels. The Golgi membrane protein 1 (GOLM1) is dysregulated in tumors [35] and currently serves as a cancer diagnostic biomarker. Interestingly, the Golgi apparatus

and mitochondria have been found to be in close contact through imaging techniques [36] yet the molecular details are unclear. The GC as a central hub coordinates multiple cellular processes, motility, growth, autophagy, apoptosis, inflammation, stress responses, and DNA repair [37]. GC-centered signaling cascades do indeed contribute to cancer. There are targets on the trans-Golgi and cis-Golgi sides [37]; starting with the trans-Golgi there are targets such as EGFR, mTORC1 that converge on SCAMP3. Wnt/B-catenin, NFκB, IRF, TLR, ERK-MAPK, JNK-MAPK converge on Sef. Ras/Raf/MEK/ERK, PI3K/AKT, VEGF/ERK all meet at RKTG/PAQR3. Caspase-8 localizes with CLIPR-59 and NK-κB at Golgin-97. Towards the Cis-Golgi, EGFR(RTK)/AKT/S6K, GSK3β/Wnt/β-catenin, PI3K/AKT/mTOR, TGF-β1/Smad2/Smad3, Notch2 all meet at GOLM1. Ras MAPK binds to UBIAD1. PI3K/AKT, GPCR/Gβγ/PI3K/ARF1/ERK, Rac1, FAK, RhoA, RhoC, pRB bind to ARF1. WNT-TCF, CNIH4/TGFα/GLI bind to TMED9. Wnt/β-catenin, HH-GLI, AKT/GSK3β/β-catenin, IL-11/STAT3, PI3K/AKT, MAPK9, JNK2 all bind to TMED3. JNK MAPK, p38 MAPK, ERK, NF-κB all bind to Ras. TFG-β/Smad, TGF-β/JNK MAPK, TGF-β/p38 MAPK, PKCδ, AMPK/mTOR can all bind to TMED10. ERK1/2 MAPK can interact with USO1/p115. GM130 on the Cis-Golgi can be a receptor for Cdc42. These are the signaling cascades that are modulated by GC-proteins. Destabilization of the classical ER-Golgi secretory pathway has been explored in the context of Golgi-targeted pharmacological approaches, and have proven effective *in vivo* and a few human cancer trials [38]. There are therapeutic advantages by selectively transporting drugs to tumor cells that are Golgi-targeted nano drug delivery systems [39]. Nanoparticles with pH-responsive photothermal ablation agent preferentially accumulate in the acidic Golgi of cancer cells [40]. There is an anti-GOLM1 antibody conjugated with pyrrolobenzodiazepine that showed antitumor response and improved survival in mice bearing xenograft tumors [41]. This is especially relevant to human gliomas and their progression through activation of AKT which is promoted by GOLM1 [42]. Mapping the complex interaction of the GC and cancer cell biology will contribute to the knowledge of this organelle in cancer.

4. Summary

The emerging field of organelle structural biology would absolutely benefit from structural studies of the Golgi complex. The ideal solution would be to use cryogenic electron tomography as it would investigate the Golgi *in situ*. Obtaining the molecular-resolution structures of the Golgi in its native environment is a step forward and considering the Golgi cisternae can be revealed by tomograms. It is important to consider the tilt series acquired when performing the capturing of these tomograms. There is an asymmetry of membrane-associated protein arrays having lateral periodicity. One could additionally execute subtomogram averaging that shows the zipper-like interactions of the trans-Golgi cisternae. One group has observed a dense granular aggregate within the cisternae and intracisternal filament bundles that were associated with trans-Golgi buds. Trafficking through the Golgi occurs via a cis to trans pathway from the ER-Golgi interaction. There is a cisternal maturation that occurs on the cis side and there are multiple transport mechanisms possible for smaller cargo. On the trans-Golgi side there are protein arrays that exclude cargo from the centers of cisternae and the cargo is promoted to exit from the periphery. The neuropathogenesis of PD includes a pathogenic fibril structure of aSyn which include polymorphs. Helical reconstruction can be performed to determine specific species like rod and twister. There are hereditary mutations in aSyn which show new polymorphs like narrow and wide fibrils. These also increase the cytotoxicity. There is also the different in rodent and human when it comes to the conformation of the mutated protein. As neurons are generated and migrate there is alterations in the neurodevelopmental process in NDDs. The secretory function can have defects and change the maturation, postmitotic neurons, and myelination. The Golgi accumulates as an outpost in dendrites which shows how the Golgi can adapt to a highly compartmentalized nature of the nervous system and the needs of the trafficking system. The ATP7A and B ion pumps located in the TGN translocate copper across intracellular membranes into the secretory pathway

to copper-dependent enzymes. It has been shows that childhood onset of dystonia and neurodegeneration whether childhood or late-onset in adults is caused by ATP7B. The neurodevelopment process was discussed from the first trimester of pregnancy to the third trimester and birth. Then after birth all the way to adolescence and the changes that occur in the nervous system as well as the Golgi. As the GC has been identified as ground zero for cancer, it too promotes the hallmarks of cancer and tumor progression. Electron microscopy has contributed greatly not only to the identification and confirmation of the Golgi but also how Golgi fragmentation is present in the biology of tumors. Evidence has been presented that the GC with cellular events is related to cancer initiation and progression. The Golgi-associated processes that can impact the cancer cell phenotype and cancer progression through regulation of cell survival/death, proliferation, motility, metabolism, and immune evasion. There is much to learn about how the Golgi conducts Ca^{2+} homeostasis and H^+ flows. GOLM1 is dysregulated in tumors and is a diagnostic biomarker. A burning question is what are the molecular details of the Golgi interactions with the mitochondrion? Many proteins and signal pathways were stated here that are modulated by GC-proteins. How one transports drugs to tumor cells targeting the Golgi through nano drug delivery systems is an active area of pharmacological research. Integrating nanoparticles with pH-responsive photothermal ablation agents the selectively accumulate in acidic Golgi of cancer cells is one way to deliver precision oncological therapeutics. Specific antibodies to a molecular target like an anti-GOLM1 are already showing antitumor responses and improved survival in animal models. This communication is to draw attention to the importance of using organelle structural biology to know more about the Golgi apparatus and how it behaves in dysfunction and disease. Using modern optical techniques like cryo-ET, multiplex CLEM, NATIVE, and SXT with computational reconstruction of the GC would provide an in-depth investigation of this organelle. The more we know about the organelle dynamics and function as it relates to these diseases could improve the mortality, morbidity, and disability of these conditions.

Funding: This research received no external funding.

Acknowledgments: D.G. thanks Professor Wah Chiu (Stanford University) and Michael Schmid (SLAC National Accelerator Laboratory) for topical and intellectual discussions about the research which lead to the generation of new ideas. Also, we would like to thank this National Center for Cryo-Electron Microscopy the Stanford-SLAC CryoEM Center (S²C²), Stanford Synchrotron Radiation Lightsource (SSRL), and the Linac Coherent Light Source (LCLS) at SLAC National Accelerator Laboratory, Stanford University.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Chabin-Brion, K., et al., *The Golgi Complex Is a Microtubule-organizing Organelle.* Molecular Biology of the Cell, 2001. **12**(7): p. 2047-2060.
2. Mollenhauer, H.H. and D.J. Morré, *Perspectives on Golgi apparatus form and function.* J Electron Microsc Tech, 1991. **17**(1): p. 2-14.
3. Rendón, W.O., et al., *Golgi fragmentation is Rab and SNARE dependent in cellular models of Parkinson's disease.* Histochem Cell Biol, 2013. **139**(5): p. 671-84.
4. Bernheimer, H., et al., *Brain dopamine and the syndromes of Parkinson and Huntington Clinical, morphological and neurochemical correlations.* Journal of the Neurological Sciences, 1973. **20**(4): p. 415-455.
5. Damier, P., et al., *The substantia nigra of the human brain: II. Patterns of loss of dopamine-containing neurons in Parkinson's disease.* Brain, 1999. **122**(8): p. 1437-1448.
6. Chaudhuri, K.R., D.G. Healy, and A.H.V. Schapira, *Non-motor symptoms of Parkinson's disease: diagnosis and management.* The Lancet Neurology, 2006. **5**(3): p. 235-245.
7. McGeer, P.L., et al., *Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains.* Neurology, 1988. **38**(8): p. 1285-91.
8. Kohutnicka, M., et al., *Microglial and astrocytic involvement in a murine model of Parkinson's disease induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP).* Immunopharmacology, 1998. **39**(3): p. 167-80.
9. Petrosyan, A., *Onco-Golgi: Is Fragmentation a Gate to Cancer Progression?* Biochem Mol Biol J, 2015. **1**(1).
10. Rios, R.M. and M. Bornens, *The Golgi apparatus at the cell centre.* Current opinion in cell biology, 2003. **15**(1): p. 60-66.
11. Eisenberg-Lerner, A., et al., *Golgi organization is regulated by proteasomal degradation.* Nat Commun, 2020. **11**(1): p. 409.

12.   Schneider, G., et al., *Three-dimensional cellular ultrastructure resolved by X-ray microscopy.* Nature methods, 2010. **7**(12): p. 985-987.

13.   Chichón, F.J., et al., *Cryo X-ray nano-tomography of vaccinia virus infected cells.* Journal of structural biology, 2012. **177**(2): p. 202-211.

14.   Duke, E.M., et al., *Imaging endosomes and autophagosomes in whole mammalian cells using correlative cryo-fluorescence and cryo-soft X-ray microscopy (cryo-CLXM).* Ultramicroscopy, 2014. **143**: p. 77-87.

15.   Yakushevska, A.E., et al., *STEM tomography in cell biology.* Journal of Structural Biology, 2007. **159**(3): p. 381-391.

16.   Barad, B.A., et al., *A surface morphometrics toolkit to quantify organellar membrane ultrastructure using cryo-electron tomography.* bioRxiv, 2022: p. 2022.01.23.477440.

17.   Polishchuk, E.V. and R.S. Polishchuk, *Chapter 15 - Analysis of Golgi Complex Function Using Correlative Light-Electron Microscopy,* in *Methods in Cell Biology,* F. Perez and D.J. Stephens, Editors. 2013, Academic Press. p. 243-258.

18.   Fang, T., et al., *Nanobody immunostaining for correlated light and electron microscopy with preservation of ultrastructure.* Nat Methods, 2018. **15**(12): p. 1029-1032.

19.   Govind, A.P., et al., *Activity-dependent Golgi satellite formation in dendrites reshapes the neuronal surface glycoproteome.* eLife, 2021. **10**: p. e68910.

20.   Antony A. Cooper, A.D.G., Anil Cashikar, Cole M. Haynes, Kathryn J. Hill, Bhupinder Bhullar, Kangning Liu, Kexiang Xu, Katherine E. Strathearn, Fang Liu, Songsong Cao, Kim A. Caldwell, Guy A. Caldwell, Gerald Marsischky, Richard D. Kolodner, Joshua LaBaer, Jean-Christophe Rochet, Nancy M. Bonini, Susan Lindquist, *a-Synuclein Blocks ER-Golgi Traffic and Rab1 Rescues Neuron Loss in Parkinson's Models.* Science, 2006.

21.   Li, B., et al., *Cryo-EM of full-length alpha-synuclein reveals fibril polymorphs with a common structural kernel.* Nat Commun, 2018. **9**(1): p. 3609.

22.   Boyer, D.R., et al., *Structures of fibrils formed by alpha-synuclein hereditary disease mutant H50Q reveal new polymorphs.* Nat Struct Mol Biol, 2019. **26**(11): p. 1044-1052.

23.   Davidson, W.S., et al., *Stabilization of alpha-synuclein secondary structure upon binding to synthetic membranes.* J Biol Chem, 1998. **273**(16): p. 9443-9.

24.   Goedert, M., *Parkinson's Disease: Alpha-Synuclein and Neurodegeneration,* in *Encyclopedia of Neuroscience,* L.R. Squire, Editor. 2009, Academic Press: Oxford. p. 461-467.

25.   Singleton, A., et al., *α-Synuclein locus triplication causes Parkinson's disease.* Science, 2003. **302**(5646): p. 841-841.

26.   Büttner, S., et al., *The Ca2+/Mn2+ ion-pump PMR1 links elevation of cytosolic Ca2+ levels to α-synuclein toxicity in Parkinson's disease models.* Cell Death & Differentiation, 2013. **20**(3): p. 465-477.

27.   Rasika, S., et al., *Golgipathies in Neurodevelopment: A New View of Old Defects.* Dev Neurosci, 2018. **40**(5-6): p. 396-416.

28.   Al-Deri, N., et al., *A novel homozygous variant in TRAPPC2L results in a neurodevelopmental disorder and disrupts TRAPP complex function.* J Med Genet, 2021. **58**(9): p. 592-601.

29.   Martinelli, D., et al., *MEDNIK syndrome: a novel defect of copper metabolism treatable by zinc acetate therapy.* Brain, 2013. **136**(Pt 3): p. 872-81.

30.   Capaci, V., et al., *Mutant p53 induces Golgi tubulo-vesiculation driving a prometastatic secretome.* Nature communications, 2020. **11**(1): p. 1-19.

31.   Li, C., et al., *THADA drives Golgi residency and upregulation of PD-L1 in cancer cells and provides promising target for immunotherapy.* Journal for immunotherapy of cancer, 2021. **9**(8).

32.   Stewart, L.M., et al., *CaMKK2 facilitates Golgi-associated vesicle trafficking to sustain cancer cell proliferation.* Cell death & disease, 2021. **12**(11): p. 1-12.

33.   Galenkamp, K.M., et al., *Golgi Acidification by NHE7 Regulates Cytosolic pH Homeostasis in Pancreatic Cancer CellsNHE7 Regulates Cytosolic pH Homeostasis in PDAC.* Cancer discovery, 2020. **10**(6): p. 822-835.

34.   Demaegd, D., et al., *Newly characterized Golgi-localized family of proteins is involved in calcium and pH homeostasis in yeast and human cells.* Proceedings of the National Academy of Sciences, 2013. **110**(17): p. 6859-6864.

35.   Liu, Y., et al., *GP73-mediated secretion of AFP and GP73 promotes proliferation and metastasis of hepatocellular carcinoma cells.* Oncogenesis, 2021. **10**(10): p. 1-12.

36.   Giorgi, C., S. Marchi, and P. Pinton, *The machineries, regulation and cellular functions of mitochondrial calcium.* Nature reviews Molecular cell biology, 2018. **19**(11): p. 713-730.

37.   Spano, D. and A. Colanzi, *Golgi Complex: A Signaling Hub in Cancer.* Cells, 2022. **11**(13).

38.   Yu, R.-Y., et al., *Regulating the Golgi apparatus by co-delivery of a COX-2 inhibitor and Brefeldin A for suppression of tumor metastasis.* Biomaterials science, 2018. **6**(8): p. 2144-2155.

39.   Zhang, M., et al., *Potential therapies and diagnosis based on Golgi-targeted nano drug delivery systems.* Pharmacological Research, 2022. **175**: p. 105861.

40.   Xue, F., et al., *A smart drug: a pH-responsive photothermal ablation agent for Golgi apparatus activated cancer therapy.* Chemical Communications, 2017. **53**(48): p. 6424-6427.

41.   Liewen, H., et al., *Therapeutic targeting of golgi phosphoprotein 2 (GOLPH2) with Armed Antibodies: A Preclinical Study of Anti-GOLPH2 antibody drug conjugates in lung and colorectal cancer models of patient derived xenografts (PDX).* Targeted Oncology, 2019. **14**(5): p. 577-590.

42.   Xu, R., et al., *PDGFA/PDGFRα-regulated GOLM1 promotes human glioma progression through activation of AKT.* Journal of Experimental & Clinical Cancer Research, 2017. **36**(1): p. 1-17.