



Beyond rare disorders

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Beyond rare disorders - a new era for peroxisomal pathophysiology

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Abstract

Metabolism is emerging as a central influencer of multiple disease states in humans. Peroxisomes are central metabolic organelles whose decreased function gives rise to severe peroxisomal diseases. It is recently becoming clear that beyond such inborn errors of metabolism, that the gradual deterioration of peroxisomal functions with age as well as small alterations in peroxisomal functions due to gene variants, contributes also to multiple and prevalent diseases such as cancer, viral infection, diabetes and neurodegeneration. Despite the clear importance of peroxisomes in pathophysiological processes, the research on peroxisomes is dramatically lagging behind research on other metabolic organelles such as mitochondria. This is perhaps due to the misconception that peroxisomes play only ancillary or redundant roles with mitochondria. This is far from true since the majority of peroxisomal functions cannot be replaced by other organelles. Here we highlight the timeliness of focusing on peroxisomes in current research on central, abundant and society-impacting human pathologies. As peroxisomes are now beginning to come into the spot light, it is clear that intensive research into these important organelles will enable a better understanding of their contribution to human health, serving as the basis to develop new diagnostic and therapeutic approaches to prevent and treat human diseases.

Introduction

Peroxisomes, named after their first ascribed function - the ability to metabolize hydrogen peroxide via catalase (Baudhuin et al., 1965; De Duve and Baudhuin, 1966) are central metabolic organelles. For approximately twenty years following their discovery, the significance of peroxisomes for human development and health remained poorly studied, until a series of independent observations in pathology and biochemistry related a set of previously described genetic disorders to defects in peroxisome function (Honsho et al., 2020; Wanders, 2018). This led to the rebranding of these inborn errors of metabolism as peroxisomal disorders and sparked an interest in the research of these organelles in relation to human pathophysiology.

Peroxisomes exist in almost all eukaryotic cells and play fundamental functions in both anabolism and catabolism of central cellular building blocks. In humans, for example, they are central in creation of ether phospholipids such as plasmalogens and take part in the synthesis of bile acids. They regulate cholesterol biosynthesis and are required for breakdown of various compounds such as D-amino acids, polyamines and a variety of fatty acids (through both α and β oxidation reactions) that cannot be broken down in mitochondria. Peroxisomes are also important for detoxification of compounds such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Figure 1) (He et al., 2021; Wanders and Waterham, 2006). Given the number of reactions that take place in peroxisomes, and their importance, it is not surprising that in their complete absence or in the absence of specific enzymatic pathways that they house, dramatic physiological effects occur. These include severe diseases such as the Zellweger spectrum disorders (ZSD), Hyperoxaluria type I, Infantile Refsum disease and Neonatal Adrenoleukodystrophy. These diseases affect multiple organs such as the kidney, eyes, liver, bone, hearing aperture and, most severely, the central nervous system. While an enormous amount of research has been carried out on these inborn errors of metabolism, we will not focus on them in this review (for comprehensive and current reviews see (Deb et al., 2021; Fujiki et al., 2020; Honsho et al., 2020))

What, however, is only recently becoming clear, is that small alterations in peroxisomal functions due to normal genetic variance and that peroxisomal deterioration with age (as is the case for all organelle functions) can contribute not only to rare disorders but also to a diversity of prevalent disease states such as cancer, viral infection, diabetes and neurodegeneration (Figure 2).

Despite their clear importance in pathophysiological processes, the research on peroxisomes is dramatically lagging behind research on other metabolic organelles such as mitochondria. This is perhaps due to the misconception that peroxisomes play only ancillary or redundant roles with mitochondria (for example both organelles can degrade ROS and fatty acids through β oxidation). However, it is becoming increasingly clear that peroxisomes are organelles whose functions cannot be replaced by other organelles making them essential for cellular wellbeing. For example, ether lipids (such as plasmalogens) that account for up to 20% of the total phospholipid content in humans and are particularly abundant in the brain, heart, and neutrophils, are produced exclusively in peroxisomes (Cipolla and Lodhi, 2017; Honsho and Fujiki, 2017). Peroxisomes are also the sole organelles in humans breaking down Very Long Chain Fatty Acids (VLCFAs) and the only ones performing α oxidation where fats are broken down via single carbons (rather than the two in β oxidation) – a process

essential for breakdown of branched chain and odd-chain length fatty acids such as phytanic acid (Wanders, 2014; Wanders et al., 2018, 2020).

The clear indications that already exist showing the role of peroxisomes in prevalent human disorders, the rapid advance in our understanding of peroxisome biology and the scarcity of research on the direct contribution of peroxisomes in multiple important human pathologies clarify that the time is now ripe to advance research of peroxisomes that goes beyond the etiology of rare disorders. Here we highlight the contribution of peroxisomes to central, abundant and society-impacting human pathologies as well as the process of aging. We foresee that in the coming decade peroxisomes will come into the spot light and that intensive research into these important organelles will enable a better understanding of their contribution to human health, serving as the basis to develop new diagnostic and therapeutic approaches to prevent and treat human diseases.

Peroxisomes in cancer

Cancer is often considered as a disease that entails aberrant metabolism. In fact, one of the hallmarks of cancer is metabolic reprogramming and plasticity which leads to a selective advantage for the initiation and progression of the malignant cells (Hanahan and Weinberg, 2011).

Peroxisomes play crucial roles in cell metabolism and it was demonstrated that almost every metabolic function that takes place in peroxisomes is related to cancer including lipid metabolism and the resulting activation of peroxisome proliferator activated receptors (PPARs), synthesis of ether phospholipids, synthesis of bile acids and ROS homeostasis (for excellent recent reviews read (Dahabieh et al., 2018; Kim, 2020). Indeed, various types of tumors exhibit alterations in peroxisome abundance and activity (Kim, 2020), and it was recently shown that the expression of peroxisomal mRNAs is elevated across different tumors (Dahabieh et al., 2018; Ravi et al., 2021). Interestingly, while some studies observed a decrease in peroxisomal activity in certain types of tumors, other reports indicate that metabolic activities of peroxisomes promote tumor growth. It is likely that the tumor-promoting or tumor-suppressing functions of peroxisomes are dependent on the tumor type and progression stage.

However, beyond metabolism, peroxisomes could contribute to tumorigenesis through their roles in apoptosis and autophagy, immunity and inflammation (Di Cara et al., 2019), non-genetic cell persistence (Shen et al., 2020) and since they have a complex interplay with other organelles (Ravi et al., 2021; Schrader et al., 2020) such as mitochondria known to have crucial roles in cancer.

Despite the critical role of peroxisomes in cell metabolism and the accumulating observations that correlate peroxisomal alterations with tumor progression, the functional effects of peroxisomes in cancer are not well recognized and not as intensively studied as those of, for example, mitochondria. Moving beyond correlations and actively exploring the causal links between peroxisomal activity and tumor progression is now an important frontier. Exploring the contribution of peroxisomes to tumorigenesis, inflammation, survival of residual cancer

cells, cell death, and other aspects of cancer may be harnessed to develop new tools to diagnose certain types of cancers and to develop new strategies to fight tumor proliferation by targeting peroxisome-related processes.

Peroxisomes in viral infection and the innate immune response

The effects of peroxisomes on viral infections as well as the contribution of peroxisomes to the innate immune response have been extensively studied in recent years leading to exciting discoveries (for comprehensive reviews summarizing the function of peroxisome in viral infections and immunity see (Di Cara et al., 2019; Cook et al., 2019; Ferreira et al., 2019, 2022)).

The understanding that peroxisomes are central for the cellular antiviral response arose from the discovery that the mitochondrial antiviral signaling protein (MAVS) is also localized to peroxisomes (Dixit et al., 2010) in addition to mitochondria, mitochondria-associated membranes (MAMs) and the endoplasmic reticulum (ER). While MAVS is found in several locals it is clear that the peroxisomal MAVS has a distinct role since activation of peroxisomal MAVS has a unique signature (Dixit et al., 2010). Follow up studies indeed demonstrated that a variety of viruses including hepatitis C virus (HCV), human cytomegalovirus (HCMV), herpes simplex virus (HSV-1), dengue virus (DENV), West Nile virus (WNV), porcine epidemic diarrhea virus (PEDV), porcine delta coronavirus (PDCoV), and even SARS-CoV-2 (Ferreira et al., 2022; Knoblach et al., 2021) affect peroxisome-mediated antiviral signaling by diverse strategies. The strategies include targeting viral proteins to the peroxisome membrane as well as affecting peroxisome biogenesis and dynamics (Ferreira et al., 2022).

Interestingly, it has also been shown that viruses do not only damage peroxisomes to escape cellular defense mechanisms, but also rewire peroxisomes to benefit from their functions. Different viruses use various strategies to manipulate peroxisome morphology and biogenesis as well as cause their re-distribution to sites of viral replication to provide building blocks. In such cases they can also manipulate peroxisome metabolism (e.g; induction of synthesis of ether lipids and downregulation of β oxidation to accumulate branched-chain or VLCFAs) to promote the formation of virus particles and viral propagation (Ferreira et al., 2022).

Importantly, the defense mechanisms that are mediated by peroxisomes are not restricted to viruses. In fact, peroxisomes were shown to also coordinate antimicrobial defenses (Di Cara et al., 2019) and influence non innate immune responses and inflammation (for a recent review read (Di Cara et al., 2019)). The contribution of peroxisomes to immunity and inflammation includes affecting the homeostasis of ROS and RNS (Fransen et al., 2012), degradation of primary modulators of inflammation (e.g; prostaglandins and leukotrienes), supplying polyunsaturated fatty acids (PUFAs) which are used as the backbone of mediators of inflammation (e.g; maresins, resolvins and protectins), affecting the metabolism of fatty acids that serve as signaling molecules of macrophages, invariant Natural Killer T (iNKT) cells, T-cells and more.

Although our understanding of the roles of peroxisomes during viral infection has dramatically expanded during the last decade, it is clear that much is still unknown about the function of

peroxisomes in different stages and types of viral infections. In times of a worldwide pandemic that enormously affected humanity, further studies of the involvement of peroxisomes in host-virus interplay have the potential to uncover delicate host-pathogen relationships and to serve as the basis to develop novel antiviral therapeutics.

Peroxisomes in obesity and diabetes

It is not surprising that a fat-degrading organelle has links to obesity and diabetes and the evidence supporting this connection is rapidly growing. Soon following the discovery of peroxisomes it was observed that adipose tissues display a large population of peroxisomes (Novikoff and Novikoff, 1982; Novikoff et al., 1980) and that adipocyte peroxisomes tend to be small in size and localized in proximity to lipid droplets (Lodhi and Semenkovich, 2014). Moreover, the number of peroxisomes was shown to increase during differentiation of adipocytes in culture (Novikoff and Novikoff, 1982). It was also demonstrated, in both cultured cells and mice, that peroxisomes affect white and brown adipocyte function and differentiation (adipogenesis) (Lodhi and Semenkovich, 2014). Moreover, mice with adipose-specific defects in peroxisomes have increased diet-induced obesity, impaired thermogenesis and decreased insulin sensitivity and glucose tolerance (Liu et al., 2019). Recently, more direct links of peroxisomes to diabetes and obesity were demonstrated. It was shown that peroxisomes are crucial to preserve the structure and function of β cells in mice and that they affect glucose tolerance, insulin secretion and cell survival (Baboota et al., 2019). In addition, it was demonstrated that peroxisomal biogenesis is downregulated in white adipose tissue (WAT) of both diet- and genetically induced obese mice causing reduced abundance of peroxisomes and suggesting a role for them in the development and progression of obesity (Piao et al., 2019). Moreover, preliminary data suggested that WATs of obese humans have lower expression of peroxisomal genes than do healthy individuals (Piao et al., 2019).

Altogether, there is a lot of correlative evidence suggesting that the deteriorating function of peroxisomes plays important roles in mediating the pathogenesis of metabolic syndromes including obesity and diabetes. However, clear causal studies are yet to emerge. Moreover, to date the vast majority of these connections was performed in cultured adipocytes with only few manuscripts looking into fat-cell functions in mice. More recently some studies have already been performed in mice models of mutated peroxins (Martens et al., 2012; Park et al., 2019, 2022) but further mice models as well as natural conditions (such as aging or diabetic diets) are still lacking. As these types of studies are undertaken, we expect that in the coming years the contribution of peroxisomes to human metabolic disorders will be unraveled and that this will open new avenues for their prevention, diagnosis and treatment.

Peroxisomes in neurodegeneration

Since many inherited peroxisomal disorders result in severe neurological phenotypes and damage stemming from defects in myelination, oxidative stress, inflammation, cell death and neuronal migration problems (Trompier et al., 2014), it stands to reason that peroxisomes are

involved in normal maintenance of brain functions and that variance in their activity as well as its decline with age, could therefore be related to neurodegeneration of aged individuals.

Since lipids constitute ~60% of the solid matter in the brain (Bourre, 2004), one of the main roles of peroxisomes in this organ is to degrade saturated VLCFAs particularly enriched in myelin. Peroxisomal β -oxidation also results in formation of docosahexaenoic acid (DHA), a central PUFA in brain and nervous tissues. In addition to its structural role in membrane lipids, DHA has been shown to act in neurotransmission, synaptic plasticity, gene expression and calcium concentration homeostasis (Salem et al., 2001). Its enzymatic conversion to resolvins, neuroprotectins and maresins provides very active anti-inflammatory molecules that can inhibit the generation of arachidonic acid oxidative derivatives (such as prostaglandins, leukotrienes, and thromboxanes) in the brain (A. Farooqui, 2012; Farooqui et al., 2007). Despite these clear connections to brain health, relatively little has been researched in regards to the role of peroxisomes in neurodegeneration. While their involvement in Alzheimer's disease as well as sclerotic diseases is evident and will also be reviewed below the exact roles of peroxisomes in neurodegeneration and in normal neuronal aging is still little understood. Moreover, while initial indications of an involvement of peroxisomal functions such as plasmalogen synthesis in other conditions such as Parkinsons' Disease are emerging (Dragonas et al., 2009; Fabelo et al., 2011; Miville-Godbout et al., 2016, 2017), a clear causal role is yet to be proven.

The role of peroxisomes in Alzheimers disease (AD)

The initial evidence for a role of peroxisomes in the development of AD was established on primary rat hippocampal neuron cultures (Santos et al., 2005). Supporting an active role of these organelles, a mouse model of AD showed significant peroxisome alterations even before disease symptoms appeared (Cimini et al., 2009). In rat, impaired peroxisome activity increased AD phenotypes at least partially by enhancing the VLCFA concentration (Shi et al., 2012). Indeed, PPAR γ agonists have been shown to ameliorate AD-related pathology in animal models of AD and improve cognition (Jo and Cho, 2019; Jo et al., 2020). There is even some indication that enhancing peroxisomal functions can be a therapeutic avenue. Treatment of primary rat neuronal cells with peroxisomally targeted catalase protects them from AD like symptoms (Giordano et al., 2014).

Most importantly, studies in humans have also correlated peroxisomal deterioration to AD progression (Kou et al., 2011). For example, several studies described a reduction of peroxisomal functions, increased VLCFAs and reduced plasmalogen and DHA levels in brain regions of AD patients that correlated with disease severity (Jo and Cho, 2019; Jo et al., 2020).

DHA reduction seems to not only be a by-product of peroxisomal dysfunction but also a driver of the disease. Indeed, its supplementation improves multiple aspects of AD pathogenesis in mice (Belkouch et al., 2016; Pan et al., 2015). Epidemiological studies further support positive DHA effects in humans (Jo and Cho, 2019; Jo et al., 2020).

The time is now ripe to use new and powerful genetic tools to cement this link, create much required insights into the direct role of peroxisomal functions in AD and utilize this knowledge to come up with potential therapeutic avenues.

Peroxisomes in Sclerosis

Anecdotal evidence exists that peroxisomes play a role in sclerotic brain events (Berger et al., 2016). For example, mutations in the peroxisomal super-oxide dismutase enzyme SOD1 (Du et al., 2018; Higgins et al., 2003; Milani et al., 2011) and in the peroxisomal enzyme D-amino acid oxidase (Mitchell et al., 2010) are associated with classical adult onset familial amyotrophic lateral sclerosis (FALS). D-amino acid oxidase overexpression in motor neurons indeed leads to activation of autophagy and cell death (Paul and De Bellerocche, 2012). There has also been observed a reduction of neuronal peroxisomes and their functions in the gray matter of patients with multiple sclerosis (MS) (Gray et al., 2014) however this is only a correlation and clearly, much more research is required to substantiate causal connections important for thinking of early detection and treatment of these diseases.

Peroxisomes in Aging

Peroxisomes contribute to many processes that have been associated with aging. One clear example is formation of free radicals that have been proposed as major drivers of aging in the free radical theory of aging (Harman, 1992). In rat liver, peroxisomes can produce up to 35% of all cellular hydrogen peroxide (Boveris et al., 1972; Giorgio et al., 2007). Another example is the peroxidation of lipids which have been assumed as major contributors to aging in the membrane theory of ageing (Zs.-Nagy, 1979). Despite this, surprisingly little has been studied in regards to the role of peroxisome function, or malfunction, in the aging process. This may be, in part, due to the similarities in the functions of peroxisomes and mitochondria, with much of the attention on oxidative-stress-associated aging focusing on the role of mitochondrial dysfunction. Despite this sparsity, there are clear indications that peroxisomes will have a major role and that this field is a *terra incognita* waiting to be discovered, explored and exploited.

The effect of aging on peroxisomes:

For many years now it has been clear that peroxisomes and their various functions are affected by aging. This is not surprising as most cellular functions studied to date indeed deteriorate with age. The first studies to have documented an age dependent decline in peroxisome functions were in rat liver (Périchon et al., 1998). Over the years accounts of multiple peroxisomal enzymes changing their activity have been reported such as a reduction in catalase activity as well as a global reduction in protein import into peroxisomes (for a summary see: Périchon et al., 1998). In human retinal epithelia of aged individuals or those suffering from age-related macular degeneration it was shown that this reduction in function seems to be counterbalanced by an increase in peroxisomes number (Feher et al., 2006). It was later shown that senescence – a major effector of aging – also correlates with reduced peroxisomal function. For example, increased catalase mislocalization is associated with the senescent state (Legakis et al., 2002). In recent years more global proteomic analysis studies of peroxisome-enriched fractions from young and old mouse liver and kidney have identified a select set of age-dependent alterations (Amelina et al., 2011; Mi et al., 2007). Similar approaches in *Caenorhabditis elegans* identified 30 peroxisomal proteins whose abundance decreased with aging (Narayan et al., 2016).

The impact of peroxisomal function on aging

Since the correlation of the decline in peroxisomal function alongside the decline in cellular activity during aging does not in any way imply causality, and since it is of importance to assign cause-effect relationships to argue that peroxisomes have a role in the aging process itself, it was of high impact on the peroxisomal field when it was shown that defects in peroxisome functions actively promote the aging process in several models. For example, in the nematode *Caenorhabditis elegans*, loss of specifically the peroxisomal catalase (but not the cytosolic one) results in the organism manifesting a progeric phenotype (Petriv and Rachubinski, 2004). Similarly, lifespan of the yeast *Saccharomyces cerevisiae* is significantly reduced when its peroxisomal catalase is knocked out (Petriv and Rachubinski, 2004). Indeed, old cells with newly established, redox-balanced peroxisomes delay appearance of senescence markers, re-establish mitochondrial integrity, and enjoy oxidative equilibrium (Koepke et al., 2007, 2008). All of these strongly suggest that by collecting more information on the direct effect of peroxisomes on aging we will gain a valuable tool in extending life and health span.

Discussion and outlook

Peroxisomes have central metabolic roles and these are emerging as affecting major diseases such as neurodegeneration, diabetes and cancer. However, we are still far from understanding which exact functions, out of the multiple ones carried out in peroxisomes, affect each cell type and disease state. We are also far from differentiating the many reports on decline or change in activity from active driving roles in disease states. While quite a lot of evidence has accumulated in model mammals and cell culture, there is little research, to date, on humans and therefore the role of peroxisomes in a huge number of diseases has not yet been elucidated. For more discussion on which directions can benefit such research see Box 1.

For example, while it is clear that peroxisomes are affected in both their number and activity during cases of ischemia-reperfusion injury (Gulati et al., 1993; Singh and Gulati, 1995) and that activation of peroxisomal fatty acid oxidation helps ameliorate the damage in such cases (Liepinsh et al., 2013) still very little has been studied regarding peroxisomes and this central disease state (Delille et al., 2006). Another example are various degenerative processes that have not been well characterized in connection to peroxisomes and these include muscular dystrophy, pulmonary emphysema and rheumatoid arthritis, (Berger et al., 2016; Masters and Crane, 1995). Most surprising is that several disease manifestations that occur in rare peroxisomal diseases have not been studied in relation to peroxisomes in individuals not carrying overt peroxisomal dysfunction and these include cataractogenesis that is a major aspect of peroxisomal disorders as well as hearing loss and kidney injury. In fact, until recently, the majority of studies on the molecular mechanisms of kidney injury focused primarily on mitochondria and neglected peroxisomes (Vasko, 2016).

The above begs the question - if peroxisomes are so important why have they not been studied intensively in relation to the above disease states until now? There are several potential reasons behind this. First, research in the last decade has focused mainly on

mitochondria in similar disease contexts and in the study of rare peroxisomal diseases. But maybe most importantly, there are a number of technical boundaries that have slowed down progress. Peroxisomes are small, highly interconnected to other organelles (Castro et al., 2018; Schrader et al., 2020) and hence difficult to purify and this has slowed down biochemical characterization in disease states. In addition, they share many central enzymatic activities with mitochondria and cytosol making it difficult to dissect the exact fraction of causality coming from peroxisomal activity. Most importantly however there seems to still be a lack of diagnostic tools both for basic research as well as for medical settings. For example, the field of mitochondrial research was revolutionized by the creation of dyes to quickly track mitochondrial number, shape and size as well as membrane potential (ie, mitotrackers). Hence, we foresee that the formation of a peroxisome-specific dye will also enable rapid assessment of peroxisomal state in multiple disease states and model systems. For more discussion on which such tools will have impact on future research see Box 2.

In the coming future we hope that the ability to genetically manipulate animal models using CRISPR/Cas9 and the powerful ability to create organoids will both enable more mechanistic insights on peroxisomal roles in disease states. As the field matures – we foresee more demonstrations of causality by reliance on mouse models of diseases and reintroduction of specific peroxisomal functions as a way to rescue disease states.

While it has taken time for peroxisomes to shine, they are now slowly taking center stage. It is clear that there is a new kid on the metabolic block and it would be wise to start taking peroxisomes more seriously and into consideration in basic, biomedical as well as translational research.

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Figure legends

Figure 1. Schematic representation of the central metabolic functions of peroxisomes in humans. Shown are the anabolic (lego) and catabolic (scissors) reactions that occur inside human peroxisomes. ROS and RNS are reactive oxygen/nitrogen species, VLCFAs are very long chain fatty acids.

Figure 2. Schematic representation of frequent human pathologies suggested to be affected by peroxisome dysfunctions.

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Box 1 Key open questions on the contribution of peroxisomes to human health and disease.

To better understand the contribution of peroxisomes to human health and to prove the causal effects of defects in peroxisomes to human pathology we foresee that the following key questions will be addressed in the coming decade: 1. What is the complete peroxisomal proteome in human cells? How is the peroxisomal proteome changed under different conditions/ in different tissues or cell types/ through disease progression and aging? 2. How is peroxisome communication and coordination with nuclear functions and other organelles regulated? What are the functions of these interactions? How do defects in the cross talk with other organelles contribute to human pathologies? 3. How are mutations and variants in peroxisomal genes and their regulatory regions associated with prevalent human pathologies? Are they more or less represented in individuals susceptible to such diseases (correlations)? Does their presence increase/reduce the susceptibility to various diseases or directly lead to the progression of such diseases (causality)?

Box 2 Tools whose development can propel peroxisome research forward and enable early diagnosis of diseases affected by altered peroxisomal functions.

We foresee that in the coming decade more research groups will study the contribution of peroxisomes to human health and that in the exome era clinicians will diagnose more patients carrying mutations in peroxisomal genes. To expand the peroxisome community and to enable early diagnosis of diseases affected by peroxisomal functions, the development of the following tools will make a significant contribution: 1. A specific live stain dye for mammalian peroxisomes to quickly track peroxisome number, shape, size and motility in live cells. 2. A simple and efficient method to purify peroxisomes. 3. Commercial antibodies for membranous and matrix peroxisomal proteins suitable for various applications (e.g; western blot, immunoprecipitation, immunostaining and immunohistochemistry). 4. Chemical compounds that can reduce or induce peroxisome number in human cells. 5. Simple kits (e.g; based on ELISA) to measure metabolic activities that take place in peroxisomes. 6. Mice models with tissue specific defects in peroxisomal genes. 7. Human cell lines with defects in peroxisomal genes (e.g; CRISPR knock out and immortalized fibroblasts from patients).



