The role of the mitochondria and the endoplasmic reticulum contact sites in the development of the immune responses

Denis Martinvalet

Abstract
Mitochondria and endoplasmic reticulum (ER) contact sites (MERCs) are dynamic modules enriched in a subset of lipids and specialized proteins that dictate both their structure and functions. The MERCs regulate lipid transfer, autophagosome formation, mitochondrial fission, Ca\(^{2+}\) homeostasis and apoptosis. Since these functions are essential for cell biology, it is therefore not surprising that MERCs also play a critical role in organ physiology among which the immune system stands by its critical host defense function. This defense system must discriminate and tolerate host cells and beneficial commensal microorganisms while eliminating pathogenic ones in order to preserve normal homeostasis. To meet this goal, the immune system has two lines of defense. First, the fast acting but unspecific innate immune system relies on anatomical physical barriers and subsets of hematopoietically derived cells expressing germline-encoded receptors called pattern recognition receptors (PRR) recognizing conserved motifs on the pathogens. Second, the slower but very specific adaptive immune response is added to complement innate immunity. Adaptive immunity relies on another set of specialized cells, the lymphocytes, harboring receptors requiring somatic recombination to be expressed. Both innate and adaptive immune cells must be activated to phagocytose and process pathogens, migrate, proliferate, release soluble factors and destroy infected cells. Some of these functions are strongly dependent on lipid transfer, autophagosome formation, mitochondrial fission, and Ca\(^{2+}\) flux; this indicates that MERCs could regulate immunity.

Facts
1. MERCs are dynamic functional modules enriched in a subset of lipids and specialized proteins that dictate both their structures and functions.
2. The activation of NLRP3 inflamasome and of MAVS-dependent antiviral response takes place at the MERCs, suggesting that these contact sites play a critical role in innate immunity.
3. MERCs play an important role in cellular Ca\(^{2+}\) homeostasis by regulating ER to mitochondrial Ca\(^{2+}\) shuttling. Since Ca\(^{2+}\) signaling is essential for lymphocyte activation, this suggests that MERCs may regulate the activation of these cells during adaptive immunity.
4. MERCs regulate both autophagy and mitochondrial fission; both processes are directly linked to antigen presentation and leukocyte migration, respectively.
5. MERCs are altered in glioma stemlike cells and consequently affect glioma stemlike cell surface glycan expression, as well as susceptibility to cytotoxic lymphocytes.

Open questions
1. What is the dynamic of the MERCs in immune cells?
2. What are the specific characteristics of immune cell MERCs?
3. Can MERCs be targeted for immune modulation?
4. MERCs are altered in glioma stemlike cells; is this a new feature of cancer stem cells?

Introduction

The endoplasmic reticulum (ER), the largest organelle in the cell, is essential for protein synthesis, folding, maturation, transport, lipid synthesis and calcium (Ca^{2+}) homeostasis. The dysregulation of the ER protein folding function triggers ER stress leading to apoptosis if not resolved^{1,4}. This tentacular ER interacts with other organelles to form membrane contact sites. At the mitochondria and ER membrane contact sites (MERCs) the two organelles are ~15–50 nm apart^{3,5–13}. The portion of membranes involved in these interactions defines the mitochondrial associated membranes (MAMs), which account for 5–20% of the mitochondrial network^{3,13,14}. MERCs are enriched in a subset of lipids and specialized proteins that dictate both their structures and functions^{14,12,15}. Moreover, the MERCs density, length and thickness depend on the cellular metabolic state and stress level, indicating that MERCs are dynamic and regulated functional units^{3,13,16,17}. Interestingly, the MERCs are crucial for lipid transfer, initiation of autophagosome formation, determination of the mitochondrial fission site, ER-mitochondria Ca^{2+} shuttling and apoptosis^{11,14,18–28}. It appears that MERCs regulate essential functions of cells biology and therefore organ physiology, among which the immune system stands by its crucial defense function.

The immune system, through its fundamental ability to distinguish self (including beneficial commensal microbiota) from non-self is able to robustly eliminate pathogenic entities and toxic molecules while preserving the integrity of the surrounding host tissues^{29–33}. To achieve its protective function, the immune system relies on anatomical physical barriers (the skin and the mucosa lining the respiratory, gastrointestinal and urogenital tracts) and a subset of hematopoietically derived cells, called leukocytes (macrophages, dendritic cells, mast cells, neutrophils, eosinophils, and natural killer (NK) cells)^{29,30}. Soluble factors, such as the complement system, pentraxins, collectins and the defensins antimicrobial peptides complete this arsenal^{29,30}. These leukocytes express a limited repertoire of germline-encoded receptors called pattern recognition receptors (PRR) recognizing conserved molecular motifs on the pathogens called pathogen associated molecular patterns (PAMPs)^{29,30,34,35}. Moreover, the PRR can also sense the damage-associated molecular pattern (DAMPs) released by host cells experiencing trauma related or not to infection^{30,35}. Altogether, these first defense lines constitute the innate immune system which is by nature fast acting but not specific^{29,30,35}. The detection of PAMPs activates tissue-resident macrophages leading to the production and secretion of the pro-inflammatory cytokine interleukin 1 (IL1) through the formation and activation of the inflammasome, a large protein complex, at the interface of the mitochondria and the ER. This indicates that MERCs play a role in the development of the innate immune response^{36–40}. Together, this leads to a state of inflammation in order to alert and to combat the ongoing infection.

Importantly, the innate immune system delivers the antigenic information to activate the adaptive immune system synergizing with the innate response. This adaptive response relies on the T lymphocytes (effectors of the cellular adaptive response) and the B lymphocytes (the antibody producing cells) harboring receptors encoded by genes requiring somatic rearrangements to be expressed^{31,41,42}. Consequently, the adaptive response takes time to build-up and comes chronologically after the innate response^{29,31}. Interestingly, the lymphocytes activation initiates a phosphorylation cascade resulting, among other things, in the mobilization of the intracellular Ca^{2+} pool essential for gene expression^{43–47}. The ignition and development of both an innate and an adaptive immune response require immune cell activation, phagocytosis and processing of pathogens, migration, proliferation, release of soluble factors, and finally, the destruction of the infected cells. Some of these functions are strongly dependent on lipid transfer, autophagosome formation, mitochondrial fission and Ca^{2+} flux indicating that MERCs could regulate immunity.

Recent excellent reviews have discussed in great detail the MERCs molecular players and functional implications for Ca^{2+} and lipid transfer, as well as the mitochondrial metabolism; therefore, this subject will not be addressed here^{4,6,15,27}. Instead, in this review, it will be put into perspective how the different function of the MERCs could impact on critical steps of both innate and adaptive immunity and see whether future work could also focus on the MERCs to regulate the host immune system.

MERCs in innate immunity

The immune system is essential for human survival as in its absence even a minor infection can be lethal^{48–50}. Once the physical barrier is breached, microbes access the host organism initiating the activation of innate immunity. Thanks to their toll-like receptors (TLR), a subfamily of PRR, patrolling macrophages are quickly alerted to the invasion^{32,51–54}, which initiates the innate immune response and brings a state of inflammation^{55}. This inflammation is triggered by the engagement and the activation of NLRP3 which belongs to the nucleotide-binding oligomerization domain-like receptors (NLRs)^{56}, a subfamily of cytosolic PRRs, particularly potent at inducing inflammation following a wide range of stimuli,
such as ATP, hyaluronan, uric acid crystal and amyloid-β. One common feature of these triggers is their ability to induce reactive oxygen species (ROS) production, suggesting that ROS are critical for NLRP3 activation. The NLRP3 activation and oligomerization recruit the adaptor protein apoptosis-associated speck-like protein containing a CARD (ASC) and procaspase 1 to form the high-molecular weight inflammasome complex. The inflammasome is in fact a platform for caspase 1 activation and caspase 1-mediated processing of pro-IL1β and IL18. The implication of ROS in NLRP3 activation suggests the involvement of MERCs in inflammation. In unstimulated cells, NLRP3 is associated with the ER, while upon activation, it redistributes to the perinuclear region at the contact site between mitochondria and the ER (Fig. 1). Moreover, oxidation of active site cysteine thiols of thioredoxin (Trx) leads to the dissociation of thioredoxin-interacting protein (TXNIP) from Trx which binds to NLRP3 and robustly activates the inflammasome. Interestingly, both ASC and TXNIP accumulate at the MERCs upon ROS-dependent activation (Fig. 1). In fact, TXNIP expression is induced by ER stress through the protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK) and inositol-requiring enzyme 1 (IRE1) pathways which enhance IL1β expression followed by its maturation through NLRP3 inflammasome. Since PERK is also a MERCs tether, whether its contribution resides in its tethering function or its ability to induce TXNIP expression needs clarification. Similarly, whether the modulation of the MERCs could hinder the activation of NLRP3 inflammasome is an interesting question that needs further consideration. In agreement with this possibility, silencing of the three human isoforms of the voltage-dependent anion channel (VDAC), another MERCs tether, severely reduced inflammasome activation and IL1β production. This result could also be explained by the contribution of VDAC in mitochondrial Ca²⁺ overload and ROS production, the latter being an agonist of NLRP3 inflammasome. VDAC is also a docking factor for mitochondria-interacting proteins, such as the metabolic switch enzyme hexokinase and the anti-apoptotic protein Bcl-2 and Bcl-XL providing the cells with both metabolic advantage and resistance to apoptosis. This resistance to cell death results from the obstruction of VDAC channel cutoff by Bcl-2 and Bcl-XL decreasing mitochondrial Ca²⁺ entry and ROS production necessary for NLRP3 inflammasome activation and IL1β production. However, VDAC also interacts with Grp75 and the inositol-1,4,5-triphosphate receptor (IP3R) to
actually physically tether the mitochondria to the ER (Fig. 1). Therefore, it is possible that silencing of VDAC also physically alters the localization and activation of the NLRP3-inflammasome at this site40.

Moreover, the identification of mitochondria antiviral-signaling protein (MAVS) as the docking site of the NLRP3 inflammasome at the MAM further confirmed the importance of MERCs in inflammation (Fig. 1)65,66. Interestingly, MAVS belongs to the mitochondrial antiviral response machinery involved in the production of type I interferons (IFNs) and pro-inflammatory cytokines67. In fact, upon infection, viral uncapped 5′-triphosphate end RNA and long double-stranded RNA are detected respectively by retinoic acid-inducible protein I (RIG-I) and melanoma differentiation-associated gene-5 (MDA-5)68–70, two cytosolic PRR with helicases and ATPase activity which discriminate the viral RNA from the abundant host RNA in the cytoplasm71,72. The binding of viral RNA triggers a conformational change in RIG I and MDA-5 that allows their interaction with MAVS at the MAM65. Their activation and translocation to the MERCs initiate signaling pathways that lead to the synthesis of multiple cytokines which include type I interferon (IFN)65,67,73,74. Interestingly, it was also reported that MAVS constitutively interacts with mitofusin 2 (MFN2), another MERCs tether, leading to the inhibition of inflammatory cytokine production. This suggests that the relocation of this antiviral response platform at the MERCs plays a complex regulatory role72,75 and as a whole, provide direct evidence of the involvement of MERCs in the establishment of a physiological inflammatory reaction as part of the innate immune response (Fig. 1 and Table 1). Further understanding of the spatiotemporal coordination of this inflammatory protein network and its regulation by MERCs is critical to therapeutically tailor inflammation as required.

**MERCs and leukocyte migration**

One key features of leukocytes is their ability to migrate throughout the organism. To illustrate this point, inflammation recruits hordes of neutrophils which massively infiltrate the site of infection to kill the invading bacteria76,77. Moreover, the activation of resident macrophages and dendritic cells by the PAMP and DAMP increases their ability to migrate to the closest draining lymph node where they will present the antigenic material to the naive B and T lymphocytes78–85. Then, these activated lymphocytes migrate to the site of infection to neutralize the infected cells; therefore, migration is a necessity for the proper function of both innate and adaptive immune cells. Interestingly, leukocyte migration requires drastic reorganization of their cytoskeleton and mitochondrial network86,87.

---

**Table 1: Immune involvement of the MERCs cell biology functions**

| Innate immunity | Adaptive immunity |
|-----------------|-------------------|
| Leukocyte migration | Leukocyte migration |
| Lymphocyte activation | Leukocyte migration |
| Sensitization to cell death, B and T cell hostostasis | Leukocyte migration |
| Mitochondrial fission | Leukocyte migration |
| Inflammation/antiviral response | Leukocyte migration |
| Autophagy | Leukocyte migration |
| Lipid transfer | Leukocyte migration |

MERCs play many essential cell biology functions, e.g., calcium signaling, cell death, mitochondrial fission, inflammation, and antiviral response, lipid transfer and autophagy that are connected to immunological processes.
Mitochondria are versatile organelles with a well-established role in cellular energy production and metabolism, Ca\(^{2+}\) homeostasis, cell cycle regulation, differentiation, cell death and aging\(^{88-93}\). Mitochondria are constantly remodeled by fusion and fission events which are regulated by a family of dynamin-related GTPases and their adaptor proteins. Mitofusin (MFN) 1 and 2 and optic atrophy 1 (OPA1) regulate outer and inner mitochondrial membrane fusion, respectively\(^{94-100}\). Mitochondrial fission is mediated by cytosolic dynamin-related protein 1 (DRP1) docking on its adaptor proteins Fis1, mitochondrial fission factor (MFF) and mitochondrial dynamics 51 and 49 kDa proteins (MiD51 and MiD49) on the outer mitochondrial membrane\(^{101-107}\). Mitochondria can respond to many cellular cues such as starvation, stress-induced depolarization and cell death\(^{94,95,98,99,106,108-116}\).

Interestingly, it was shown that during leukocyte migration there is a redistribution of the mitochondria at the cell uropods in a mitochondrial fission- and calcium-dependent manner\(^{96,87,117,118}\). Mitochondrial fission facilitates their relocation and promotes lymphocyte chemotaxis, whereas mitochondrial division inhibits both processes probably due to the inability to transport too large organelles along the cytoskeleton\(^{96,87,117-119}\). Interestingly, both mitochondrial fission and cellular Ca\(^{2+}\) homeostasis are regulated by MERCs (Figs. 1, 2 and Table 1)\(^{11,14,18,20,22,27}\). At the MERCs defined by the ER tubules wrapping the mitochondria, the mitochondria are constricted\(^{3,11}\). It was also proposed that these MERCs provide a platform for the recruitment of motor generating force cytoskeletal proteins\(^3\). In fact, ER-bound inverted formin 2 (INF2) concentrates between the two organelles where ER wraps the mitochondria (Fig. 1)\(^{3,20,120}\). The INF2 triggers the assembly of the actomyosin motor providing the force for the initial constriction of the mitochondria\(^{3,11,20,120}\). Once assembled, the ER-associated constricted mitochondria enable polymerized DRP1 to spiral around the mitochondria to mediate their fission\(^{3,11,20,101,102,106,120}\). Moreover, mitochondrial movement along microtubules is regulated by calcium oscillation-dependent Miro-Milton complex interaction with kinesin motor\(^{3,117,121-124}\). Interestingly, Miro is an outer mitochondrial membrane protein enriched at the MERCs\(^{123,125}\). It interacts with dynein through the cytosolic factor Milton giving molecular insight into how calcium regulates the mitochondrial redistribution that also occurs during cell migration\(^{122,126,127}\).

Similarly, MERCs also play an important role in intracellular calcium homeostasis. Actually, the regulation of mitochondrial calcium uptake is the best described MERCs function so far. In resting condition, Ca\(^{2+}\) level ranges from nanomolar to micromolar concentration in the cytosol and the mitochondria, respectively, while it is in the half millimolar range in the ER\(^{93}\). This asymmetric calcium distribution is tightly regulated by a variety of calcium channels, pumps and exchangers expressed at the plasma membrane, the ER, and the mitochondria\(^{14,22,43,46,47,128-134}\). The coordination of the different calcium pools relies on membrane contact sites between the ER and the plasma membrane, and between the ER and mitochondria, thus acting as signaling platforms to ensure synchronized activities of Ca\(^{2+}\) channels, pumps and exchangers\(^{3,12,43,46,47,128,131-134}\). Upon activation, phospholipase C (PLC) produces inositol-1,4,5-triphosphate (IP3) from plasma membrane phosphatidylinositol-4,5 diphasphate\(^{135,136}\). The IP3 triggers ER Ca\(^{2+}\) release through a channel formed at the ER membrane by the IP3 receptor (IP3R)\(^{135,136}\). This IP3R-mediated ER calcium release is buffered by mitochondrial Ca\(^{2+}\) uptake through VDAC and the mitochondrial Ca\(^{2+}\) uniporter (MCU), located in the outer and inner mitochondrial membrane, respectively (Fig. 1)\(^{131,132}\). Both IP3R and VDAC are concentrated at ER-mitochondria contact sites where they also contribute to tether these two organelles together (Fig. 1)\(^3,12\). The 75 kDa glucose-regulated protein (GRP75) interacts with both IP3R and VDAC to reinforce and increase the coupling of these two ion channels (Fig. 1)\(^3,12,137\). This ER-mitochondria Ca\(^{2+}\) shuttling is regulated by a supramolecular weight protein complex including AKT kinase, promyelocytic leukemia (PML) and the serine threonine phosphatase PP2A enriched in the MAM\(^{138-140}\). Moreover, the vesicle-associated membrane protein-associated protein B (VAPB), an integral ER protein whose amino-terminus projects into the cytosol, interacts with the outer mitochondrial membrane protein tyrosine phosphatase-interacting protein 51 (PTPPIP51) to favor the ER-mitochondria Ca\(^{2+}\) exchange (Fig. 1)\(^{141}\). The apposition of the ER with the mitochondria at these contact sites forms a confined space enabling to build-up local Ca\(^{2+}\) microdomains. These Ca\(^{2+}\) microdomains reach concentration compatible with MCU low affinity in order to ensure mitochondrial calcium uptake. This mitochondrial calcium uptake is necessary for activation of TCA dehydrogenase involved in ATP production and in the regulation of cell death\(^{128,142-146}\). Together, this indicates that MERCs, by their ability to regulate mitochondrial fission and calcium homeostasis, can modulate leukocyte migration and function (Table 1 and Fig. 2).

**MERCs and antigen presentation**

Dendritic cells (DCs), macrophages and B cells are professional antigen presenting cells (APC) as they excel in the ability to ingest and process antigenic material to present in the context of their major histocompatibility complex class I (MHC-I) or class II (MHC-II) molecules in order to activate cytotoxic CD8\(^{+}\) T cells or CD4\(^{+}\) helper T cells, respectively\(^{78,79,147-149}\). The mechanism of
antigen presentation has been reviewed in detail elsewhere. Conventionally, MHC-I molecules are normally loaded with peptides derived from cytosolic proteolysis, while MHC-II molecules are loaded with peptides from extracellular pathogens that have been phagocytosed. When DCs are directly infected with viruses, they generate MHC-I antigenic peptides by the classical pathway. However, in situations where the DCs are not directly infected or in case of tumors and allogeneic transplants, the antigens are internalized by phagocytosis of microbes, infected, allogeneic or transformed dying cells and cross-presented on the MHC-I of the DCs. Interestingly, autophagy potentiates both MHC-I, MHC-II antigen presentation and MHC-I cross-presentation, the latter being seen when the autophagic process is triggered in donor cells. Autophagy is also critical for the survival, differentiation and function of T lymphocytes and therefore plays an important role in the immune response. There are at least three different types of autophagy, including macroautophagy (usually simply referred as autophagy), chaperone-mediated autophagy and micro-autophagy. Autophagy is the process by which cytosolic components and organelles are segregated in a double membrane compartment, the autophagosome, for degradation and recycling following autophagosome fusion with lysosomes. Nutrient deprivation is a potent inducer of autophagy through the inhibition of the mammalian target of rapamycin (mTOR). This leads to the activation and relocation of mTOR substrates ULK1/2, ATG13 and FIP200 from the cytosol to certain domains of the ER and the subsequent recruitment of the class III
phosphatidylinositol kinase (class III-P13K) complex VPS34/VPS15/beclin 1 and ATG14 to the ER [163,167–171]. The autophagosome formation also requires ATG12-ATG5 and the phosphatidylethanolamine (PE)-conjugated ATG8/LC3, GATE16, and GABARAP two ubiquitin-like conjugating systems [163,172–176]. It is suggested that the ER is crucial for the initiation of the autophagosome formation which takes place at the contact site between the ER and the mitochondria [19,177]. Moreover, upon starvation, the pre-autophagosome marker ATG14 is redistributed at MERCs while ATG5 also localizes at this site until autophagosome formation is completed [19]. Strikingly, starvation also triggers an increase in MERCs length [17]. Whether this increase in MERCs length could be a mechanism to modulate autophagy and the dependent antigen presentation needs to be addressed. Taken together, the critical importance of autophagy during antigen presentation could suggest that MERCs are likely critical regulators of lymphocyte activation as they contribute to autophagosome biogenesis (Table 1 and Fig. 2).

MERCs and lymphocyte activation

Engagement of the B-cell receptor (BCR) or the T-cell receptor (TCR) and their respective co-receptors triggers an intracellular phosphorylation cascade culminating in the activation of the transcription factors API, NF-κB, NFAT, OCA-B/OBF-1 and Pip/IRF-4 that are critical for B and T lymphocyte activation, respectively [31,178–182]. One common feature of lymphocyte activation is the recruitment and activation of phospholipase Cγ (PLCγ) (Fig. 3) [31,179,180] which mediates the production of IP3 and diacylglycerol (DAG). The DAG activates protein kinase C (PKC) initiating a phosphorylation cascade ending with the activation of the transcription factor NF-κB (Fig. 3) [183–186]. The IP3 induces a rapid increase in intracellular Ca2+ levels by means of activation of stores operating calcium (SOCl) influx following IP3R-mediated ER calcium store release and activation of calcium-release activated calcium (CRAC) channel [185,186,133–136]. Cytosolic Ca2+ binds to calmodulin to activate the phosphatase calcineurin. Active calcineurin/calmodulin complex dephosphorylates and activates the nuclear factor of activated T cells (NFAT) [31,44]. Since intracellular Ca2+ flux is essential for lymphocyte activation, any biological processes impeding on this Ca2+ homeostasis can impact lymphocyte activation. For example, phosphoenol pyruvate (PEP), by repressing the activity of sarco/ER Ca2+-ATPase (SERCA) whose function is to pump Ca2+ back into the ER, sustains TCR-mediated Ca2+-NFAT signaling and potentiates T cell activation [187,188]. Interestingly, mitochondria buffer the inflowing Ca2+ and prevent the blunting of the CRAC Ca2+ allowing full activation of T cells [87,189]. As discussed earlier, MERCs are particularly involved in the modulation of the cellular Ca2+ homeostasis; this suggests that MERCs most likely play a critical role in modulating lymphocytes activation (Table 1 and Figs. 2, 3). As already stated, mitochondrial Ca2+ overload sensitizes cells to death (Fig. 2 darkened zone of the calcium signaling segment) [22,146,189–193]. Furthermore, the interaction of the outer mitochondrial membrane protein Fis1 with the ER protein Bap31 constitutes an additional MERC tether, whose function is to provide a platform for apoptosis induction (Fig. 1) [28,194–196]. This suggests that MERCs could also act on lymphocyte homeostasis by regulating their development during negative selection and their elimination after antigen-dependent peripheral expansion in the process called activation induced cell death (AICD) (Table 1 and Fig. 2). Both processes involve massive lymphocyte death [42,197–199].

MERCs and the cytotoxic anticancer response

The role of the immune system against cancer was only demonstrated as primary and acquired immunodeficiency are associated with increased susceptibility to cancer [200–203]. Furthermore, the size of the immune infiltrate in primary tumor is a good prognosis for patient survival, explaining why blockade of immune checkpoint receptors CTLA4 and PD-1 is a very promising immunotherapy strategy [202,204–208]. Further supporting the importance of the anti-tumoral immunity, it was shown that among 810 mutant mouse lines screened to identify microenvironmental regulators of metastatic colonization, only 23 genes were important among which 19 have immune function [209]. Nevertheless, the occurrence of cancers is a direct demonstration that tumor cells are capable of evading the immune surveillance [202,210–213]. This ability to escape immune recognition and elimination is now a new hallmark of cancer [214].

Strikingly, cancer cell plasma membrane topography and glycocalyx regulate the ability of cytotoxic lymphocytes to contact them [215,216]. To trigger apoptosis, cytotoxic lymphocytes must form an immunological synapse with their cancer target cells into which they degranulate their cytotoxic granule content [16,179,217–225]. We found this pathway to also be dependent on target cell mitochondrial ROS production [219–222,224]. Using a glioblastoma multiform model, a very aggressive primary malignant brain tumor, we found that surface glycan expression regulates these cancer cells engagement by cytotoxic lymphocytes [225,226]. In fact, the glioma stemlike cells (GSC) which expressed lower surface sialylated glycans were more susceptible to cytotoxic lymphocytes as opposed to the glioma differentiated cells (GDC) (Fig. 4) [226–231]. Compared to GDC, DRP1 expression was higher in GSC, while MFN2 expression was reduced, explaining their fragmented mitochondrial
phenotype\textsuperscript{226,232}. MFN2 was the only MERCs tether whose expression was significantly reduced in GSC as opposed to GDC\textsuperscript{28,137,141,233,234}, in agreement with GSC shorter mitochondria poorly interacting with the ER, than did the elongated mitochondria in GDC. Consequently, after stimulation, GSC experienced reduced mitochondrial Ca\textsuperscript{2+} uptake as compared to their GDC counterparts. Excitingly, restoring the ER-mitochondria contact in GSC with an artificial tether was sufficient to restore the surface expression of certain
sialylated glycans and reduce their susceptibility to cytotoxic lymphocyte-mediated killing (Fig. 4)226.

Protein and lipid glycosylation are critical for cell physiology235–237. Changes in glycosylation and the expression level of surface sialic acid and sialyltransferase are directly correlated with tumor metastasis238–240. Interestingly, MAM is enriched in glycosyltransferases and ceramide synthase activities241–243. Moreover, in addition to the vesicular transport system, the exchanges and the biosynthesis of lipids also require MERCs3,21,244,245. In yeast, the ERMES complex at the MERCs is composed of Mmm1, Mdm10, Mdm12, and

Fig. 4 Mitochondria morphology and dynamism regulate glioma surface glycan expression and sensitivity to cytotoxic lymphocytes mediated killing. On the left, glioma differentiated cells (GDC) have reticulated long mitochondria that interact well with the ER to form MERCs, leading to a high-surface expression of some glycans, impeding engagement and killing by cytotoxic lymphocytes. On the right, glioma stemlike cells have short and highly dynamic mitochondria that make less MERCs, resulting in a lower surface expression of glycans, better engagement and killing by cytotoxic lymphocytes. ER, endoplasmic reticulum; Mito, mitochondria
Mdm34 that are functionally connected to phospholipid biosynthesis21,244,246. During their biosynthesis, lipids commute back and forth between ER and mitochondria at membrane contact sites. In the ER, phosphatidic acid is converted to phosphatidylserine which is further decarboxylated in the mitochondria inner membrane to form phosphatidylethanolamine. Phosphatidylethanolamine shuttles back to the ER where it is transformed into phosphatidylcholine3,12,23,247. Therefore, it is likely that the MERCs defects observed in GSC could result in an altered biosynthesis or bioavailability of lipids, essential for the surface expression of some glycolipids. In future studies, it would be very important to test whether MERCs dysregulation is a novel feature of cancer stemlike cells regardless of their histological origin.

**MERCs and human diseases**

MERCs may be a novel regulatory hub for cancer development through the recruitment of proto-oncogenes and tumor suppressor15. Indeed, extra nuclear accumulation of the tumor suppressor PML at the MERCs, where it forms a supramolecular weight complex with PP2A and AKT, provides a regulatory module for the ER-mitochondrial Ca$^{2+}$ transfer apparatus140,248. This localization of a pool of PML is essential for the propagation of apoptotic stimuli following mitochondrial Ca$^{2+}$ overload in conditions of cellular stress140,248. Similarly, PTEN, another tumor suppressor, is also enriched at MAM where it also regulates AKT-dependent phosphorylation of IP3R and ER-mitochondria Ca$^{2+}$ shuttling. Interestingly, at the MERCs, mTORC2 interacts with the IP3R/Grp75/VDAC1 to regulate not only MAM's integrity but also mitochondrial ATP production in a manner that not surprisingly touches many other organelles, including the mitochondria. Mitochondria-ER contact sites (MERCs) play critical functions, such as lipid transfer, initiation of autophagosome formation, determination of the mitochondrial fission site, mitochondrial Ca$^{2+}$ homeostasis, as well as apoptosis11,14,18–28. As we have seen, many of these functions are important for both innate and adaptive immunity. Actually, because of the different challenges immune cells must face to protect the organism from invading pathogens, they represent good models to further investigate the MERCs structurally, biochemically, and functionally. These investigations are expected to provide additional important insight into the role of these dynamic membrane interactions in the pathophysiology of the immune system.

**Conclusion**

The necessity to isolate biochemical reactions impose the cell compartmentalization with the consequence of restricting the mutualisation of essential constituents. To overcome this restriction, the different cell compartments communicate at a contact zone, defined as organelle membrane contact sites. The ER is the largest cell network that not surprisingly touches many other organelles, including the mitochondria. Mitochondria-ER contact sites (MERCs) play critical functions, such as lipid transfer, initiation of autophagosome formation, determination of the mitochondrial fission site, mitochondrial Ca$^{2+}$ homeostasis, as well as apoptosis11,14,18–28. As we have seen, many of these functions are important for both innate and adaptive immunity. Actually, because of the different challenges immune cells must face to protect the organism from invading pathogens, they represent good models to further investigate the MERCs structurally, biochemically, and functionally. These investigations are expected to provide additional important insight into the role of these dynamic membrane interactions in the pathophysiology of the immune system.

**Acknowledgements**

This work was supported by a Subsidy from Foundation Privée des HUG, ERC starting grant ERC-2010-StG_20091118 and Foundation Boninchi. I thank Dr. Jerome Thiery for proofreading this manuscript.

**Conflict of interest**

The author declares that they have no conflict of interest.
References

1. Gardner, B. M. & Walter, P. Unfolded proteins are Ire1-activating ligands that directly induce the unfolded protein response. Science 333, 1891–1894 (2011).

2. Lu, M. et al. Opposing unfolded protein-response signals converge on death receptor S to control apoptosis. Science 345, 98–101 (2014).

3. Phillips, M. L. & Voeltz, G. K. Structure and function of ER membrane contact sites with other organelles. Nat. Rev. Mol. Cell Biol. 17, 69–82 (2016).

4. Gorgi, C. et al. Mitochondria-associated membranes: composition, molecular mechanisms, and physiopathological implications. Antioxid. Redox Signal 22, 995–1019 (2015).

5. Ciordas, G. et al. Structural and functional features and significance of the physical linkage between ER and mitochondria. J. Cell Biol. 174, 915–921 (2016).

6. Gorgi, C., De Stefani, D., Bononi, A., Rizzuto, R. & Pinton, P. Structural and functional link between the mitochondrial network and the endoplasmic reticulum. Int. J. Biochem. Cell Biol. 41, 1817–1827 (2009).

7. Mannella, C. A., Buttle, K., Rath, B. K. & Marko, M. Electron microscopic tomography of rat-liver mitochondria and their interaction with the endoplasmic reticulum. Biochimica 8, 225–229 (1998).

8. Moyer, P. J., Szycher, M. A. & Meyer, U. A. Isolation and characterization of rough endoplasmic reticulum associated with mitochondria from normal rat liver. Biochem. Biophys. Acta 646, 283–297 (1981).

9. Shore, G. C. & Tata, J. R. Two fractions of rough endoplasmic reticulum from rat liver. I. Recovery of rapidly sedimenting endoplasmic reticulum in association with mitochondria. J. Cell Biol. 72, 714–725 (1977).

10. Ogata, T. & Yamaiaki, Y. Ultra-high-resolution scanning electron microscopy of mitochondria and sarcoplasmic reticulum arrangement in human red, white, and intermediate muscle fibers. Anat. Rec. 248, 214–223 (1997).

11. Friedmann, J. R. et al. ER tubules mark sites of mitochondrial division. Science 334, 358–362 (2011).

12. Helle, S. C. et al. Organization and function of membrane contact sites. Biochim. Biophys. Acta 1833, 2526–2541 (2013).

13. Giaccomelli, M. & Pellegrini, L. The coming of age of the mitochondria-ER contact: a matter of thickness. Cell Death Differ. 23, 1417–1427 (2016).

14. Rizzuto, R. et al. Close contacts with the endoplasmic reticulum as determinants of mitochondrial Ca2+ responses. Science 280, 1763–1766 (1998).

15. van Vijver, A. R., Verfaillie, T. & Agostinis, P. New functions of mitochondria associated membranes in cellular signaling. Biochim. Biophys. Acta 1843, 2253–2262 (2014).

16. Bravo, R. et al. Increased ER-mitochondrial coupling promotes mitochondrial respiration and bioenergetics during early phases of ER stress. J. Cell Sci. 124, 2143–2152 (2011).

17. Sood, A. et al. Mitofusin-2-dependent inactivating cleavage of Opa1 links changes in mitochondria cristae and ER contacts in the postprandial liver. Proc. Natl Acad. Sci. USA 111, 16017–16022 (2014).

18. Rowland, A. A. & Voeltz, G. K. Endoplasmic reticulum-mitochondria contacts function of the junction. Nat. Rev. Mol. Cell Biol. 13, 607–625 (2012).

19. Hamasaki, M. et al. Autophagosomes form at ER-mitochondria contact sites. Nature 495, 389–393 (2013).

20. Kordoba, F., Ramabadran, V. & Higgs, H. N. An actin-dependent step in mitochondrial fission mediated by the ER-associated formin INF2. Science 339, 464–467 (2013).

21. Kornmann, B. et al. An ER-mitochondria tethering complex revealed by a synthetic biology screen. Science 325, 477–481 (2009).

22. Rizzuto, R., Birni, M., Murgia, M. & Pozzan, T. Microdomains with high Ca2+ close to sensitive channels that are sensed by neighboring mitochondria. Science 262, 744–747 (1993).

23. Stone, S. J. & Vance, J. E. Phosphatidylethanolamine synthase-1 and -2 are localized to mitochondria-associated membranes. J. Biol. Chem. 275, 34534–34540 (2000).

24. Picconi, M. et al. FAC14, a new gene encoding long-chain acyl-CoA synthetase 4, is deleted in a family with Alport syndrome, elliptocytosis, and mental retardation. Genomics 47, 350–358 (1998).

25. Ruzin, A. E., Cui, Z., Chen, N. H. & Vance, J. E. A unique mitochondria-associated membrane fraction from rat liver has a high capacity for lipid synthesis and contains pre-Golgi secretory proteins including nascent lipoproteins. J. Biol. Chem. 269, 27494–27502 (1994).

26. Thomson, M. Does cholesterol use the mitochondrial contact site as a conduit to the steroidogenic pathway? Bioessays 25, 252–258 (2003).

27. Hayashi, T., Rizzuto, R., Hajnoczky, G. & Su, T. P. MAM: more than just a housekeeper. Trends Cell Biol. 19, 81–88 (2009).

28. Iwai, K. et al. IRE1alpha induces thioredoxin-interacting protein to activate the NLRP3 inflammasome and promote programmed cell death under irremediable ER stress. Cell Metab. 16, 250–264 (2012).

29. Marchi, S., Paternagni, S. & Pinton, P. The endoplasmic reticulum-mitochondria connection: one touch, multiple functions. Biochim. Biophys. Acta 1837, 461–469 (2014).

30. Saxena, G., Chen, J. & Shalev, A. Intracellular shuttling and mitochondrial function of thioredoxin-interacting protein. J. Biol. Chem. 285, 3997–4005 (2010).

31. Zhou, R., Tardivel, A., Thorens, B., Choi, I. & Tschopp, J. Thioredoxin-interacting protein links oxidative stress to inflammasome activation. Nat. Immunol. 11, 136–140 (2010).

32. Zhou, R., Yachi, A. S., Menu, P. & Tschopp, J. A role for mitochondria in NLRP3 inflammasome activation. Nature 469, 221–225 (2011).

33. Alder, M. N. et al. Diversity and function of adaptive immune receptors in a jawless vertebrate. Science 310, 1970–1973 (2005).

34. Wardemann, H. et al. Predominant autoantibody production by early human B cell precursors. Science 301, 1374–1377 (2003).

35. Fei, S. et al. A mutation in Oral1 causes immune deficiency by abrogating CRAC channel function. Nature 491, 179–185 (2006).

36. Hogan, P. G., Chen, L., Nardone, J. B. & Rao, A. Transcriptional regulation by calcium, calcineurin, and NFAT. Genes Dev. 17, 2205–2232 (2003).

37. Kummerow, C. et al. The immunological synapse controls local and global calcium signals in T lymphocytes. Immunol. Rev. 231, 132–147 (2009).

38. Yeromin, A. V. et al. Molecular identification of the CRAC channel by altered ion selectivity in a mutant of Oral. Nature 443, 229–226 (2006).

39. Zhang, S. L. et al. STIM1 is a Ca2+ sensor that activates CRAC channels and migrates from the Ca2+-store to the plasma membrane. Nature 437, 902–905 (2005).

40. Cavazana-Calvo, M. et al. Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease. Science 288, 669–672 (2000).

41. Buckley, R. H. et al. Hematopoietic stem-cell transplantation for the treatment of severe combined immunodeficiency. N. Engl. J. Med. 340, 508–516 (1999).

42. Markert, M. L. et al. Transplantation of thymus tissue in complete DiGeorge syndrome. N. Engl. J. Med. 341, 1180–1189 (1999).

43. Alexopoulou, L., Holt, A. C., Medzhitov, R. & Flavell, R. A. Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. Nature 413, 72–76 (2001).

44. Hemmi, H. et al. A Toll-like receptor recognizes bacterial DNA. Nature 408, 745–749 (2000).

45. Termeer, C. et al. Oligosaccharides of Hylauronan activate dendritic cells via toll-like receptor 4. J. Exp. Med. 195, 99–111 (2002).
54. Poltorak, A. et al. Defective LPS signaling in C3H/HeJ and C57Bl/10ScCr mice: mutations in Tlr4 gene. Science 282, 2086–2088 (1998).

55. Newton, K. D, Voelz, V. M. Signaling in innate immunity and inflammation. Cold Spring Harb. Perspect. Biol. 4. 2012.

56. Schroder, K. & Tschopp, J. The inflammasomes. Cell 140, 812–832 (2010).

57. Mariani, S. et al. Cryopyrin activates the inflammasome in response to toxins and ATP. Nature 440, 228–232 (2006).

58. Yamazaki, K. et al. NLPR3/cryopyrin is necessary for interleukin-1beta (IL-1beta) release in response to hyaluronan, an endogenous trigger of inflammasome in response to injury. J. Biol. Chem. 284, 12762–12771 (2009).

59. Halle, A. et al. The NAIP3 inflammasome is involved in the innate immune response to amyloid-beta. Nat. Immunol. 9, 857–865 (2008).

60. Cruz, C. M. et al. ATP activates a reactive oxygen species-dependent oxidative stress response and secretion of proinflammatory cytokines in macrophages. J. Biol. Chem. 282, 2871–2879 (2007).

61. Dostert, C. et al. Innate immune activation through NAIP3 inflammasome sensing of asbestos and silica. Science 320, 674–677 (2008).

62. Oslowski, C. M. et al. Thioredoxin-interacting protein mediates ER stress-induced beta cell death through initiation of the inflammasome. Cell 16, 265–273 (2012).

63. Arbel, N. & Shoshan-Barmatz, V. Voltage-dependent anion channel 1-based peptides interact with B2 to prevent antipapoptotic activity. J. Biol. Chem. 285, 6053–6062 (2010).

64. Shoshan-Barmatz, V. & Miazchi, D. VDAC1: from structure to cancer therapy. Front. Oncol. 2, 164 (2012).

65. Horner, S. M. et al. Mitochondrial-associated endoplasmic reticulum membrane (MAM) form innate immune synapses and are targeted by hepatitis C virus. Proc. Natl Acad. Sci. USA 108, 14950–14955 (2011).

66. Subramanian, N. Tataran, K. I., Clayworth, M. W., Wang, Z. & Germain, R. N. The adaptor MAVS promotes NLRP3 mitochondrial localization and inflammasome activation. Cell 153, 348–361 (2013).

67. Seth, R. B., Sun, L., El, C. K. & Chen, Z. J. Identification and characterization of MAVS, a mitochondrial antiviral signaling protein that activates NF-kappaB and IRF 3. Cell 122, 669–682 (2005).

68. Hornung, V. et al. S-Tryptophosphate RNA is the ligand for RIG-I. Science 314, 994–997 (2006).

69. Pichlmair, A. et al. RIG-I-mediated antiviral responses to single-stranded RNA bearing 5'-triphosphates. Science 314, 997–1001 (2006).

70. Galluzzi, L., Kepp, O. & Kroemer, G. Mitochondria master regulators of danger signaling. Nat. Rev. Mol. Cell Biol. 13, 780–788 (2012).

71. Luo, Y. M. & Gale, M. J. Jr. Immune signaling by RIG-I-like receptors. Immunity 34, 680–692 (2011).

72. Pourcelot, M. & Arnoult, D. Mitochondrial dynamics and the innate antiviral immune response. FEBS J. 281, 3791–3802 (2014).

73. Kawai, T. & Akira, S. Toll-like receptor and RIG-I-like receptor signaling. Cell 126, 1137–1150 (2006).

74. Kawai, T. & Akira, S. RIG-I-like receptors in mammalian immunity. Annu. Rev. Immunol. 28, 281–315 (2010).

75. Akira, S. NLRs and the inflammasome. Science 325, 43–48 (2009).

76. Kawai, T. & Akira, S. RIG-I-like receptors in mammalian immunity. Annu. Rev. Immunol. 28, 247–279 (2010).
118. da Silva, A. F., Mariotti, F. R., Maximo, V. & Campello, S. Mitochondria dynamics by the Miro-Milton complex. Cell Mol Life Sci. 71, 2313–2324 (2014).
119. Hollenbeck, P. J. & Saxton, W. M. The axonal transport of mitochondria. J. Cell Biol. 118. (2002).
120. Tanaka, Y. et al. Targeted disruption of mouse conventional kinesin heavy chain, kif5B, results in abnormal perinuclear clustering of mitochondria. J. Cell Sci. 118, 1451–1456 (2011).
121. Glatzer, E. E., Megeath, L. J., Stowers, R. S. & Schwarz, T. L. Axonal transport of mitochondria requires miton to recruit kinesin heavy chain and is light chain independent. J. Cell Biol. 173, 545–557 (2006).
122. Stowers, R. S., Megeath, L. J., Gonka-Andrejajek, J., Meinertzhagen, I. A. & Schwarz, T. L. Axonal transport of mitochondria to synapses depends on miton, a novel Drosophila protein. Neuron 36, 1063–1077 (2002).
123. Nunes, P. & Dernaurex, N. Redox regulation of store-operated Ca2+ release. Antioxid. Redox Signal. 21, 915–932 (2014).
124. Ben-Kasius Nissim, T. et al. Mitochondria control store-operated Ca2+ entry through Na+/Ca2+ exchange. Biochemistry 50, 797–817 (2011).
125. Bogeski, I. et al. Differential redox regulation of ORAI ion channels: a mechanism to tune cellular calcium signaling. Sci. Signal. 3, ra124 (2010).
126. De Stefani, D., Raffaello, A., Teardo, E., Szabo, I. & Rizzuto, R. A forty-kilodalton protein of the inner membrane is the mitochondrial calcium uniporter. Nature 476, 336–340 (2011).
127. Baughman, J. M. et al. Integrative genomics identifies MCU as an essential component of the mitochondrial calcium uniporter. Nature 476, 341–345 (2011).
128. Piair, M. et al. Orai1 is an essential pore subunit of the CRAC channel. Nature 443, 230–233 (2006).
129. Vg, M. et al. CRACM1 is a plasma membrane protein essential for store-operated Ca2+ entry. Science 312, 1220–1223 (2006).
130. Etoh, S. & Hama, K. IP3 receptor Ca2+ channel: from discovery to new signaling concepts. J. Neurochem. 102, 1426–1446 (2007).
131. Putney, J. W. Jr. A model for receptor-regulated calcium entry. Cell Calcium 7, 1–12 (1986).
132. Szabadai, G. et al. Chaperone-mediated coupling of endoplasmic reticulum and mitochondrial Ca2+-channels. J. Cell Biol. 175, 901–911 (2006).

133. MacAskill, A. F. et al. Miro1 is a calcium sensor for glutamate receptor-dependent localization of mitochondria at synapses. J. Cell Sci. 122, 1158–1168 (2009).
134. MacAskill, A. F. et al. Miro1 is a calcium sensor for glutamate receptor-dependent localization of mitochondria at synapses. J. Cell Sci. 122, 1158–1168 (2009).
135. Putney, J. W. Jr. A model for receptor-regulated calcium entry. Cell Calcium 7, 1–12 (1986).
136. Putney, J. W. Jr. A model for receptor-regulated calcium entry. Cell Calcium 7, 1–12 (1986).
137. MacAskill, A. F. et al. Miro1 is a calcium sensor for glutamate receptor-dependent localization of mitochondria at synapses. J. Cell Sci. 122, 1158–1168 (2009).
138. Marchi, S. et al. Selective modulation of subtype III IP3R2 by Akt regulates ER Ca2+(i) release and apoptosis. Cell Death Dis. 3, e304 (2012).
139. Marchi, S. et al. Akt kinase reducing endoplasmic reticulum Ca2+release protects cells from Ca2+-dependent apoptotic stimuli. Biochem. Biophys. Res. Commun. 375, 501–505 (2008).
140. Gorgi, C. et al. PML regulates apoptosis at endoplasmic reticulum by modulating calcium release. Science 330, 1247–1251 (2010).
141. De Vos, K. J. et al. VAPB interacts with the mitochondrial protein PTPP1 to regulate calcium homeostasis. Hum. Mol. Genet. 21, 1299–1311 (2012).
142. Denton, R. M., McCormack, J. G. & Edgell, N. J. Role of calcium ions in the regulation of intramitochondrial metabolism. Effects of Na+, Mg2+ and ruthenium red on the Ca2+-stimulated oxidation of oxoglutarate and on cytochrome oxidase activity in intact rat heart mitochondria. Biochem. J. 190, 107–117 (1980).
143. McCormack, J. G. & Denton, R. M. Role of calcium ions in the regulation of intramitochondrial metabolism. Properties of the Ca2+-sensitive dye rhod-2 and rhod-2-AM. Biochem. J. 190, 107–117 (1980).
144. Denton, R. M., Richards, D. A. & Chin, J. G. Calcium ions and the regulation of NAD+ -linked isocitrate dehydrogenase from the mitochondria of rat heart and other tissues. Biochem. J. 176, 899–906 (1978).
145. Sciamanna, L. et al. BAX and BAK regulation of endoplasmic reticulum Ca2+ -a control point for apoptosis. Science 300, 135–139 (2003).
146. Hjorungnes, A. et al. Mitochondrial calcium signals and cell death: approaches for assessing the role of mitochondrial Ca2+ uptake in apoptosis. Cell 40, 553–560 (2006).
147. Ziegler, H. K. & Unanue, E. R. Decrease in macrophage antigen catabolism caused by ammonia and chloroquine is associated with inhibition of antigen presentation to T cells. Proc. Natl Acad. Sci. USA 79, 175–178 (1982).
148. Mantegazza, A. R., Magalhaes, J. G., Amigorena, S. & Marks, M. S. Presentation of phagocytosed antigens by MHC class I and II. Traffic 14, 135–152 (2013).
149. Hildner, K. et al. Baf3 deficiency reveals a critical role for CD11balpha+ dendritic cells in cytotoxic T cell immunity. J. Exp. Med. 227, 227–236 (2013).
150. Genovese, M. et al. Autophagy within the antigen donor cell facilitates efficient antigen cross-presentation in CD8(+) dendritic cells. J. Immunol. 182, 3335–3341 (2009).
151. Hussmann, H. et al. Alternative endogenous protein processing via an autophagy-dependent pathway compensates for Yersinia-mediated inhibition of endosomal major histocompatibility complex class II antigen presentation. Infect. Immun. 78, 5138–5150 (2010).
152. Thurston, T. L., Rydahl, G., Bloor, S., von Muhlenin, N. & Randow, F. The TBI1 adaptor and autophagy receptor NDP52 restricts the proliferation of ubiquitin-coated bacteria. Nat. Immunol. 10, 1215–1221 (2009).
153. Yano, T. et al. Autophagic control of listeria through intracellular innate immune recognition in drosophila. Science 322, 1097–1100 (2008).
154. Crozier, V. L. & Blum, J. S. Autophagy and its role in MHC-mediated antigen presentation. J. Immunol. 182, 3335–3341 (2009).
155. Dangel, J. et al. Autophagy promotes MHC class II presentation of peptides from intracellular source proteins. Proc. Natl Acad. Sci. USA 102, 7922–7927 (2005).
156. Ma, Y., Galluzzi, L., Zitvogel, L. & Kroemer, G. Autophagy and cellular immune responses. Immunity 39, 211–227 (2013).
157. English, L. et al. Autophagy enhances the presentation of endogenous viral antigens on MHC class I molecules during HSV-1 infection. Nat. Immunol. 10, 480–487 (2009).
158. Li, Y. et al. Efficient cross-presentation depends on autophagy in tumor cells. Cancer Res. 68, 6889–6895 (2008).
159. Uhl, M. et al. Autophagy within the antigen donor cell facilitates efficient antigen cross-presentation of virus-specific CD8+ T cells. Cell Death Differ. 16, 991–1005 (2009).
160. Jr, W., Jr, C., Jr & Jr, W. Jr. Autophagy regulates endoplasmic reticulum homeostasis and calcium mobilization in T lymphocytes. J. Immunol. 186, 1564–1574 (2011).
161. Pau, H. H., Dzhagalov, I., Chuck, M., Mizumura, N. & Jr, W. Jr. Critical role for the autophagy gene Atg5 in T cell survival and proliferation. J. Exp. Med. 204, 25–31 (2007).
162. Levine, B., Mizumura, N. & Jr, W. Jr. Autophagy in inflammation and infection. Nature 459, 323–335 (2011).
Weidberg, H. et al. LC3 and GATE-16/GABARAP subfamilies are both essential

Axe, E. L. et al. Autophagosome formation from membrane compartments

Fujita, N. et al. An Atg4B mutant hampers the lipidation of LC3 paralogues

Mizushima, N. The role of the Atg1/ULK1 complex in autophagy regulation.

Itakura, E. & Mizushima, N. Characterization of autophagosome formation site by a hierarchical analysis of mammalian Atg proteins. Autophagy 6, 764–776 (2010).

Matsunaga, K. et al. Two Toc1 binding proteins, Atg14L and Rubicon, reciprocally regulate autophagy at different stages. Nat. Cell Biol. 11, 385–396 (2009).

Axe, E. L. et al. Autophagosome formation from membrane compartments enriched in phospholipid/most 3-phosphate and dynamically connected to the endoplasmic reticulum. J. Cell Biol. 182, 685–701 (2008).

Kistakis, N. T., Andrews, S. & Long, J. What is the advantage of a transient precursor in autophagosome biogenesis? Autophagy 7, 118–122 (2011).

Fujita, N. et al. An Atg4B mutant hampers the lipidation of LC3 paralogues and causes defects in autophagosome closure. Mol. Cell Biol. 19, 4651–4659 (2009).

Fujita, N. et al. The Atg16L complex specifies the site of LC3 lipidation for membrane biogenesis in autophagy. Mol. Cell Biol. 19, 2092–2100 (2008).

Itoh, T. et al. Golgi-resident small GTPase Rab33B interacts with Atg16L and modulates autophagosome formation. Mol. Biol Cell 19, 2916–2925 (2008).

Weidberg, H., Shpakova, T., Shvets, E. & Elazar, Z. Mammalian autophagy: one is simply not enough. Autophagy 6, 808–809 (2010).

Weidberg, H. et al. LC3 and GATE-16/GABARAP subfamilies are both essential yet act differently in autophagosome biogenesis. EMBO J. 29, 1792–1802 (2010).

Hamasaki, M., Shibutani, S. T. & Yoshimori, T. Up-to-date membrane biogenesis in the autophagosome formation. Curr. Opin. Cell Biol. 25, 455–460 (2013).

Kim, U. et al. The B-cell-specific transcription coactivator OCA-B/OBF-1/Rob-1 is essential for normal production of immunoglobulin isotypes. Nature 383, 542–547 (1996).

Huppa, J. B., Geimer, M., Sumen, C. & Davis, M. M. Continuos T cell receptor signaling required for synapse maintenance and full effector potential. Nat. Immunol. 4, 749–755 (2003).

Chen, L. et al. Expression of ZAP-70 is associated with increased B-cell membrane immunoglobulin levels. J. Immunol. 170, 4600–4614 (2003).

Schulz, D. B., Rolink, A., Kosco-Vilbois, M. H., Botten, F. & Matthias, P. A B-cell-specific coactivator OBF-1/OCA-B/Rob1 required for immune response and germinal centre formation. Nature 383, 538–542 (1996).

Brass, A. L., Zhu, A. Q. & Singh, H. Assembly requirements of PU.1-IP (IRF-4) activator complexes: inhibiting function in vivo using fused dimers. EMBO J. 18, 977–991 (1999).

Nishizuka, Y. Protein kinase C and lipid signaling for sustained cellular responses. FASEB J. 9, 484–496 (1995).

Teddler, T. F. & Engel, P. CD20, a regulator of cell-cycle progression of B lymphocytes. Immunol. Today 15, 450–454 (1994).

Teddler, T. F., Zhou, L. J. & Engel, P. The CD19/CD21 signal transduction complex of B lymphocytes. Immunol. Today 15, 437–442 (1994).

Tuwshin, S. A. et al. Protein kinase Calpha (PKalpha) acts upstream of PI3Kbeta to activate Ikapba kinase andNF-kappaB in T lymphocytes. Mol. Cell 45, 797–808 (2011).

Hop, P. C. et al. Phosphoehorlase is a metabolic checkpoint of anti-tumor T cell responses. Cell 162, 1217–1228 (2015).

Chang, C. H. et al. Metabolic competition in the tumor microenvironment is a driver of cancer progression. Cell 162, 1229–1241 (2015).

Parekh, A. B. & Putney, J. W. Jr. Store-operated calcium channels. Physiol. Rev. 92, 857–8106 (2002).

Battori, G., Ciodras, G., Garcia-Perez, C., Davies, E. & Hajnoczky, G. Ca2+-dependent control of the permeability properties of the mammalian outer membrane and voltage-dependent anion-selective channel (VDAC) J. Biol. Chem. 281, 17347–17358 (2006).
229. Singh, S. K. et al. Identi...
227. Chen, J. et al. A restricted cell population propagates glioblastoma growth...
228. Suva, M. L. et al. Reconstructing and reprogramming the tumor-propagating...
224. Martinvalet, D. & Thiery, J. A novel caspase-independent apoptotic pathway...
220. Jacquemin, G. et al. Granzyme B-induced mitochondrial ROS are required for apoptosis. Cell Death Differ. 22, 862–874 (2015).
221. Martinvalet, D., Dykhooom, D. M., Ferrini, R. & Lieberman, J. Granzyme A cleaves a mitochondrial complex I protein to initiate caspase-independent cell death. Cell 133, 681–692 (2008).
222. Martinvalet, D., Zhu, P. & Lieberman, J. Granzyme A induces caspase-independent mitochondrial damage, a required first step for apoptosis. Immunity 22, 355–370 (2005).
223. Thiery, J. et al. Perforin pores in the endosomal membrane trigger the release of endocyotzed granzyme B into the cytosol of target cells. Nat. Immunol. 12, 770–777 (2011).
224. Martinvalet, D. & Thiery, J. A novel caspase-independent apoptotic pathway triggered by Granzyme B. A Med. Sci. 24, 901–903 (2008).
225. Castro, M. G. et al. Current and future strategies for the treatment of malignant brain tumors. Pharmacol. Ther. 98, 71–108 (2003).
226. Bassoy, E. Y. et al. ER-mitochondria contacts control surface glycan expression and sensitivity to killer lymphocytes in gloma stem-like cells. EMBO J. 36, 1493–1512 (2017).
227. Chen, J. et al. A restricted cell population propagates glioblastoma growth after chemotherapy. Nature 488, 522–526 (2012).
228. Suva, M. L. et al. Reconstructing and reprogramming the tumor-propagating potential of glioblastoma stem-like cells. Cell 157, 580–594 (2014).
229. Singh, S. K. et al. Identification of human brain tumour initiating cells. Nature 432, 396–401 (2004).
230. Patel, A. P. et al. Single-cell RNA-seq highlights intratumoral heterogeneity in primary glioblastoma. Science 344, 1396–1401 (2014).
231. Bassoy, E. Y. et al. Glioma stem-like cells enhance the killing of glioma differentiated cells by cytotoxic lymphocytes. Proc. Natl. Acad. Sci. U.S.A 111, e0153433 (2016).
232. Xie, Q. et al. Mitochondrial control by DRP1 in brain tumor initiating cells. Nat. Neurosci. 18, 501–510 (2015).
233. de Brito, O. M. & Scorrano, L. Mitofusin 2 tethers endoplasmic reticulum to mitochondria. Nature 456, 605–610 (2008).
234. Naon, D. et al. Critical reappraisal confirms that Mitofusin 2 is an endoplasmic reticulum-mitochondria tether. Proc. Natl. Acad. Sci. U.S.A 113, 11249–11254 (2016).
235. Kleene, R. & Schachner, M. Glycans and neural cell interactions. Nat. Rev. Neurosci. 5, 195–208 (2004).
236. Ohtsubo, K & Marth, J. D. Glycosylation in cellular mechanisms of health and disease. Cell 126, 855–867 (2006).
237. Tuccillo, F. M. et al. Aberrant glycosylation as biomarker for cancer: focus on CD43. Biomed. Res. Int. 2014, 742831 (2014).
238. Kannagi, R., Itazawa, M., Kolle, T., Miyazaki, K. & Kimura, N. Carbohydrate-mediated cell adhesion in cancer metastasis and angiogenesis. Cancer Sci. 95, 377–384 (2004).
239. Dmitroff, C. J. et al. Cell surface α-acetyleneuraminic acidalpha2,3-galactoside-dependent intercellular adhesion of human colon cancer cells. Biochem. Biophys. Res. Commun. 256, 631–636 (1999).
240. Fuster, M. M., Brown, J. R., Wang, L. & Esko, J. D. A disaccharide precursor of sialyl Lewis X inhibits metastatic potential of tumor cells. Cancer Res. 63, 2775–2781 (2003).
241. Ardali, D. et al. The mitochondria-associated endoplasmic-reticulum subcompartment (MAM fraction) of rat liver contains highly active sphingolipid-specific glycosyltransferases. Biochem. J. 371, 1013–1019 (2003).
242. Bionda, C., Portoukalian, J., Schmitt, D., Rodriguez-Lafresse, C. & Ardali, D. Subcellular compartmentalization of ceramide metabolism: MAM (mitochondria-associated membrane) and/or mitochondria? Biochem. J. 382, 527–533 (2004).
243. Mullens, T. D., Hanunn, Y. A. & Obeid, L. M. Ceramide synthases at the centre of sphingolipid metabolism and biology. Biochem. J. 441, 789–802 (2012).
244. Kornmann, B. & Walter, P. ER/EMES-mediated ER-mitochondria contacts: molecular hubs for the regulation of mitochondrial biology. J. Cell Sci. 123, 1389–1393 (2010).
245. De Mario, A., Quintana-Cabrera, R., Martinvalet, D. & Giacomello, M (Neuro) degenerated Mitochondria-ER contacts. Biochem. Biophys. Res. Commun. 483, 1096–1109 (2016).
246. Hirabayashi, Y. et al. ER-mitochondria tethering by PDZD8 regulates Ca2+ dynamics in mammalian neurons. Science 358, 623–630 (2017).
247. Vange, J. E. Phospholipid synthesis in a membrane fraction associated with mitochondria. J. Biol. Chem. 265, 7248–7256 (1990).
248. Salomoni, P. & Pandolfi, P. P. The role of PML in tumor suppression. Cell 108, 165–170 (2002).
249. Betz, C. et al. Feature article: mTOR complex 2-Akt signaling at mitochondria-associated endoplasmic reticulum membranes (MAM) regulates mitochondrial physiology. Proc. Natl. Acad. Sci. U.S.A 110, 12526–12534 (2013).
250. Rature, A. & Simmen, T. Where the endoplasmic reticulum and the mitochondria tie the knot: the mitochondria-associated membrane (MAM). Biochim. Biophys. Acta 1833, 213–224 (2013).
251. Simmen, T. et al. PACS-2 controls endoplasmic reticulum-mitochondria communication and Bid-mediated apoptosis. EMBO J. 24, 717–729 (2005).
252. Masui, S., Vavassori, S., Fagioli, C., Sitia, R. & Inaba, K. Molecular bases of cyclic flow driving actomyosin contractility on matrix. J. Cell Sci. 126, 921–929 (2013).
253. Vange, J. E. Phospholipid synthesis in a membrane fraction associated with mitochondria. J. Biol. Chem. 265, 7248–7256 (1990).
254. Dimitroff, C. J. et al. Cell surface α-acetyleneuraminic acidalpha2,3-galactoside-dependent intercellular adhesion of human colon cancer cells. Biochem. Biophys. Res. Commun. 256, 631–636 (1999).
255. Fuster, M. M., Brown, J. R., Wang, L. & Esko, J. D. A disaccharide precursor of sialyl Lewis X inhibits metastatic potential of tumor cells. Cancer Res. 63, 2775–2781 (2003).
256. Ardali, D. et al. The mitochondria-associated endoplasmic-reticulum subcompartment (MAM fraction) of rat liver contains highly active sphingolipid-specific glycosyltransferases. Biochem. J. 371, 1013–1019 (2003).
257. Vange, J. E. M. A. M. (mitochondria-associated membranes) in mammalian cells: lipids and beyond. Biochem. Biophys. Acta 1841, 595–609 (2014).