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Lysosomal exocytosis: From cell protection to protumoral functions

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ABSTRACT

Lysosomes are single membrane bounded group of acidic organelles that can be involved in a process called lysosomal exocytosis which leads to the extracellular release of their content. Lysosomal exocytosis is required for plasma membrane repair or remodeling events such as bone resorption, antigen presentation or mitosis, and for protection against toxic agents such as heavy metals. Recently, it has been showed that to fulfill this protective role, lysosomal exocytosis needs some autophagic proteins, in an autophagy-independent manner. In addition to these crucial physiological roles, lysosomal exocytosis plays a major protumoral role in various cancers. This effect is exerted through tumor microenvironment modifications, including extracellular matrix remodeling, acidosis, oncogenic and profibrogenic signals. This review provides a comprehensive overview of the different elements released in the microenvironment during lysosomal exocytosis, i.e. proteases, exosomes, and protons, and their effects in the context of tumor development and treatment.

Lysosomes were first discovered in the 1950s by C. De Duve et al., a Belgian biochemist and his team while they were studying glucose-6-phosphatase and insulin action in the liver [1]. Lysosomes are single membrane bounded group of acidic organelles, containing two classes of proteins, necessary for their main digestive and recycling functions: soluble hydrolases and integral lysosomal membrane proteins [2,3]. Luminal proteins include various hydrolytic enzymes such as proteases, sulfatases, lipases, phosphatases, nucleases and glycosidases, which have been the subject of excellent reviews [4,5]. Lysosomes digestion substrates are taken from the extracellular space through endocytosis or phagocytosis and from the cytoplasm by autophagy [6]. Lysosomes vary in shape, size, content and intracellular location, forming a heterogeneous organelles group [7]. When located close to the plasma membrane, lysosomes can carry out a process known as lysosomal exocytosis. Lysosomal exocytosis is a ubiquitous and generalized process first described in the release of acid hydrolases in the Howship lacuna of osteoclasts during bone resorption [8]. This mechanism is essential for various physiological process, such as plasma membrane repair and remodeling, ATP and H⁺ release, immune response and antigen presentation [9]. In this review, we focus on molecular mechanisms of lysosomal exocytosis and the role of this process, with a particular

emphasis in cancer.

1. 1- Lysosomal exocytosis: molecular mechanism

Lysosomal exocytosis is a multi-step process (Fig. 1). Lysosome transport from the MTOC (microtubule organizing center) in perinuclear location, to the cell periphery is a prerequisite to exocytosis, and is mediated by kinesin motors [10]. Kinesins bind microtubules by a globular motor domain and lysosomes by a tail domain through specific adaptor or cargos [11]. Several studies showed that lysosomes interact with different kinesins for this centrifugal transport, but the best characterized are KINESIN-1 [10,12]. The multi-subunit complex BORG (BLOC one-related complex) is associated with the cytosolic face of lysosomes, and activate the ADP-ribosylation Factor Like GTPase 8B (ARL8B) and its effector SifA and kinesin-interacting protein (SKIP), whose recruit Kinesin-1 and -3 to enhance anterograde transport of lysosomes [13–17]. Alternatively, the Rab (Ras-related protein) small GTPase Rab7 and its effector FYVE and coiled-coil [CC] domain containing 1 (FYCO1) in combination with PI(3)P (phosphatidylinositol 3 phosphate) can recruit Kinesin-1 for centrifuge lysosomal transport [13]. Lysosomal-Associated Membrane Protein 1 (LAMP1) is a

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lysosomal transmembrane protein which contains a large, glycosylated/sialylated N-terminal luminal domain and a short C-terminal cytosolic tail [18,19]. LAMP1 could also be involved in lysosome anterograde traffic through its direct interaction with motor myosin-11 [20]. Indeed, myosins are other components of motors proteins which, while kinesins move fast along microtubule, move slower on actin filaments. Rab small GTPases have also been involved in this process. A complex formed by Rab3a, the guanine nucleotide exchange factor for Rab3a (GRAB) and Rab11, together with synaptotagmin-like protein 4a (SLP4-a) and non-muscle myosin heavy chain IIA is required, as LAMP1, for lysosome positioning at the cell periphery and lysosome exocytosis [21,22]. Other proteins or parameters such as the deubiquitinase ubiquitin-specific peptidase 17 (USP17) or membrane tension gradients have recently been involved in lysosome positioning at the cell periphery [23,24]. Now close to the plasma membrane, lysosomes have to dock and fuse to the plasma membrane. These processes require (i) an important cytoplasmic Ca^{2+} increase, and (ii) interactions between several lysosomal and plasma membrane proteins [25,26]. Ca^{2+} release, from lysosomes or endoplasmic reticulum, is mediated by nuclear translocation of Transcription Factor EB (TFEB), which promotes the transcription of Transient Receptor Potential Mucolipin 1 (TRPML1) [25,26]. Indeed, TRPML1 is a non-selective lysosomal membrane channel, permeable to various cations and playing a positive role in lysosomal exocytosis process by supplying Ca^{2+} for membrane fusion [27,28]. In addition, TRPML1 activation on lysosomes triggers Ca^{2+} release endoplasmic reticulum [25]. The silencing of TRPML1 inhibits lysosomal exocytosis, and human mutations in TRPML1 result in type IV mucopolidosis (ML-IV), a childhood neurodegenerative lysosomal storage disorder [29,30]. Synaptotagmin VII (SYT VII) is a lysosomal

membrane protein with a single transmembrane domain and a cytosolic main part. SYT VII plays a key role in lysosomal exocytosis and membrane fusion as a Ca^{2+} sensor. TRPML1-mediated Ca^{2+} efflux or Ca^{2+} influx following plasma membrane wounds leads to SYT VII conformational change. This promotes its interaction with the V-SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) protein VAMP7, at the surface of the lysosome and with the T-SNARE-E-phospholipid interacting complex formed by SYNTAXIN-4 and SNAP23, (synaptosome-associated protein of 23 kDa) at the plasma membrane [31]. This complex molecular mechanism leads to docking of lysosomes to the plasma membrane (Fig. 1), lysosomal and plasma membrane fusion, extracellular release of lysosomal luminal content and exposure of LAMP1 at the plasma membrane, which is commonly used as a marker of lysosomal exocytosis [32].

Although the role of luminal enzymes in the onset and regulation of lysosomal exocytosis remains to be elucidated, neuraminidase 1 (NEU1) has been identified as a negative regulator of lysosomal exocytosis. Lysosomal exocytosis regulation is complex and ensured by numerous players that will not be described here. However, NEU1 inactivation results in excessive lysosomal exocytosis and represents a useful model to analyze this process [9,20,33]. NEU1 is the most abundant of the 4 mammalian sialidases and is ubiquitously expressed. During lysosomal exocytosis, NEU1 can perform a hydrolytic removal of the sialic acids on LAMP1, thus limiting the number of lysosomes that dock to the plasma membrane, and thereby lysosomal exocytosis [34,35]. NEU1 activity requires the cathepsin A protein and together with beta-galactosidase, these 3 hydrolases composed the megadalton-sized lysosomal multi-complex enzyme [36]. A inherited genetic defect in one of these components leads to lysosomal storage diseases resulting from NEU1 or

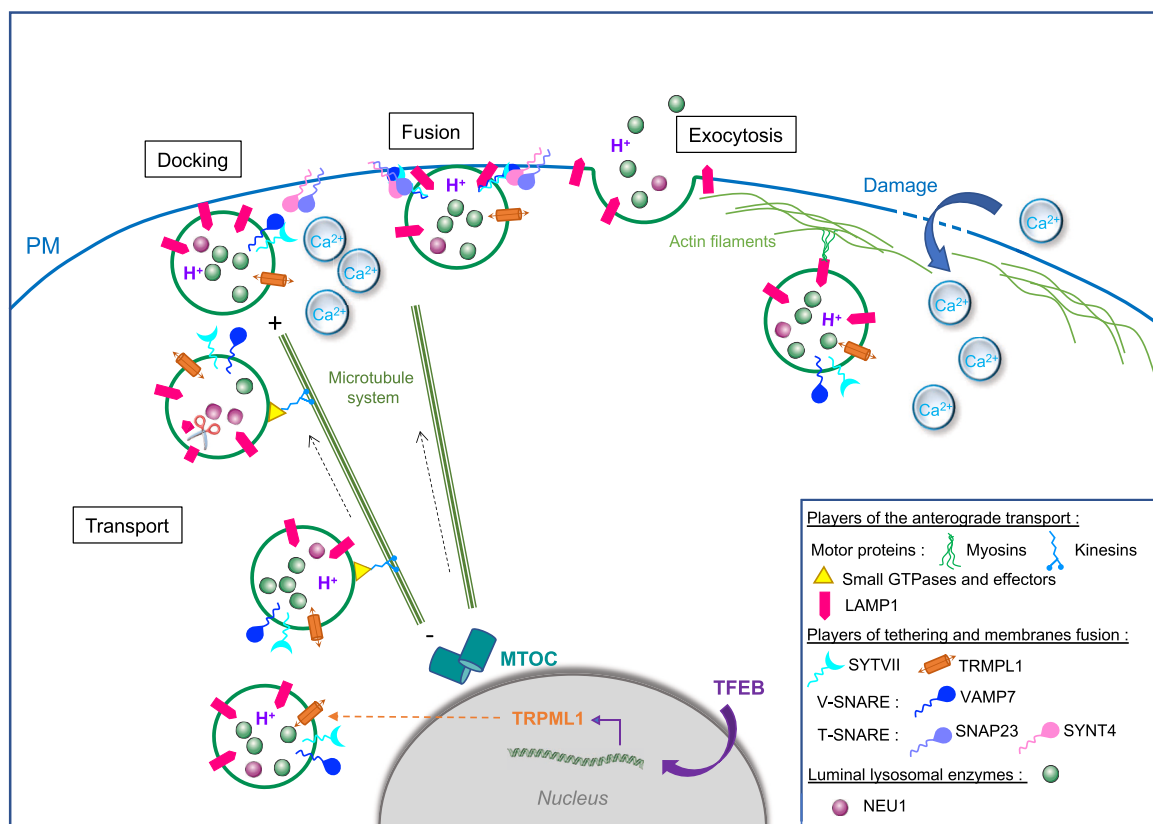


Fig. 1. Schematic representation of the molecular mechanisms leading to lysosomal exocytosis (Adapted from Machado et al., 2021).

The main steps of the process and the major actors are represented. First perinuclear lysosomes traffic to plasma membrane along the microtubule system. Second, LAMP1 mediates lysosomal docking and fusion of lysosome to the plasma membrane. The required Ca^{2+} efflux induces SYT VII conformational change and its interaction with V-SNARE and T-SNARE complex. Exocytosis results in the extracellular release of lysosomal luminal content and exposure of LAMP1 at the plasma membrane. The process can be downregulated by NEU1, which removes sialic acids on LAMP1 and thus limits the docking step.

beta-galactosidase activity defect and called sialidosis galactosialidosis [37].

2. 2- Lysosomal exocytosis: non-cancer roles

Lysosomal exocytosis exerts several critical functions such as plasma membrane repair, plasma membrane remodeling, cell protection against toxic products or cytotoxicity at the immunological synapse (Fig. 2).

2.1. Plasma membrane repair

Damage to the plasma membrane results from various insults and its repair is an emergency response required for cell survival. The involvement of lysosomal exocytosis in plasma membrane repair (PMR) was first shown by Reddy and al. in NRK cells following scratch-wounding and by analyzing the presence of LAMP1 at the cell surface or by blocking the process using SYT VII and LAMP1 inhibitors [27]. Afterwards, several authors confirmed and extended the role of lysosomal exocytosis in PMR after membrane disruption due to various stresses such as pathogens or pore-forming proteins in different cell types [38–40]. Interestingly, lysosomal exocytosis plays also an important role in PMR through the extracellular release of its content as it has been shown with the acid sphingomyelinase (ASM) [41]. Indeed, ASM is a luminal lysosomal hydrolase secreted in the extracellular compartment during lysosomal exocytosis. Experimentally, purified ASM promotes endocytosis and wound removal when added extracellularly and rescues the PMR defect of ASM-deficient cells [41]. Similarly, lysosomal cysteine proteases cathepsin B and L which are quickly secreted in the case of membrane injury, are needed for wound repair [42]. In addition to its role in PMR, lysosomal exocytosis can also be involved in plasma membrane remodeling.

2.2. Plasma membrane/extracellular microenvironment remodeling

Several cellular crucial processes involve membrane remodeling/rearrangement. Bone resorption, antigen presentation and mitosis are presented hereafter as examples of the lysosomal exocytosis involvement in these processes.

2.2.1. Bone resorption

Bone homeostasis requires a balance between bone formation by osteoblasts and bone resorption by osteoclasts [43]. Osteoclastic resorption involves osteoclast adhesion to the bone matrix, formation of an actin ring to seal the specific zone of resorption and generation of a specialized membrane called the “ruffled border” due to the fusion of lysosomes at the apical site of the plasma membrane. This process leads to the formation of a resorption lacunae in which the matrix is dissolved according to the following steps: (a) acidification of the lacunae, notably via the lysosomal V-ATPase activity, leading to dissolution of the mineral phase; (b) degradation of the protein matrix by collagenases and lysosomal enzymes. Thus, the constant secretion of protons and lysosomal proteases in the lacunae is required for bone resorption [44,45]. As previously mentioned, lysosomal exocytosis and lysosomal enzyme release were first described for their key role in osteoclasts during bone resorption [8]. Later, it has been observed that lysosomal enzymes such as cathepsin C and β -glucuronidase were localized along the osteoclast ruffled border and in the extracellular bone-resorbing compartment, in particular between the finger-like projections of the ruffled border [46]. Then, it has been shown *in vitro* that SYT VII is associated with lysosomes in osteoclasts, and the absence of SYT VII inhibits cathepsin K secretion and the formation of the ruffled border, suggesting a major role of lysosomal exocytosis in bone resorption [47]. These data emphasize the intimate story of bone resorption and lysosomal exocytosis.

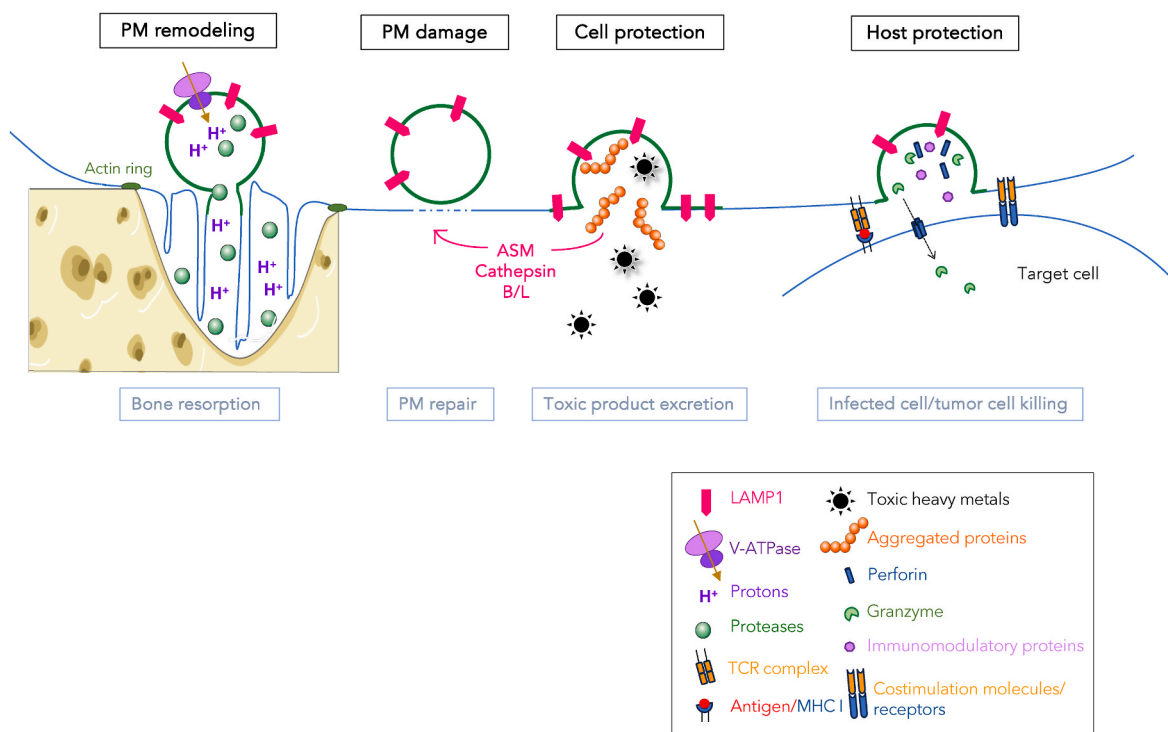


Fig. 2. Non-cancer roles of lysosomal exocytosis.

Lysosomal exocytosis can be involved in plasma membrane (PM) remodeling represented here by ruffled border formation in the case of bone resorption by osteoclasts. Lysosomal exocytosis is also crucial for plasma membrane repair. Lysosomal membrane is used for plasma membrane resealing and some lysosomal proteins such as acid sphingomyelinase (ASM) and cathepsins B and L are involved in wound repair. Lysosomal exocytosis is also involved in cellular clearance of toxic products such as heavy metals or aggregated proteins. Finally, lysosomal exocytosis plays a crucial role in the immunological synapse by releasing several proteins such as perforin and granzyme to kill the target cell.

2.2.2. Antigen presentation

In addition to its role in the degradation of pathogens or harmful cells by phagocytes [48], lysosomal exocytosis was shown to be involved in antigen presentation [49,50]. B lymphocytes induce antibody responses that neutralize pathogens. Antigen encountered by B cells is often associated with the surface of pathogens and/or antigen-presenting cells. B-cells are able to extract and internalize these surface-associated antigens [51]. This requires antigen-B cell receptor (BCR) interaction leading to antigen internalization, followed by an intracellular processing and surface presentation to T cells. BCR-mediated recognition of surface-associated antigen induces localized plasma membrane permeabilization, which triggers plasma membrane repair/remodeling involving lysosomal exocytosis. Lysosomal exocytosis results in the releasing of hydrolases that can cleave antigen from surfaces, facilitating internalization and presentation to T-cells [49,50].

2.2.3. Mitosis

Mitosis involves extensive cell membrane remodeling activities including an increase of cell surface area. This process involves a “membrane donor” which is not well defined. In their study, Nugues et al. demonstrate that pharmacological or genetic inhibition of lysosomal exocytosis induces mitotic failure, suggesting that lysosomal exocytosis is required for mitosis progression [52].

2.3. Cell protection against toxic products

2.3.1. Heavy metal protection

Lysosomal exocytosis is also involved in heavy metal protection. Indeed, iron overload-associated cytotoxicity can be reverted through TFEB expression and associated lysosomal exocytosis, which is dependent on a TRPML1-mediated increase in cytosolic calcium levels [53]. Similarly, copper increase over a threshold value induces lysosomal exocytosis and copper excretion from the cells [54,55]. Moreover, cells exhibiting high lysosomal exocytosis showed a decreased Zinc intracellular content leading to transcriptional reprogramming [56]. Finally, in the uranium-exposed nephrotoxicity mice model, a specific TRPML1 agonist significantly decreases uranium accumulation in the kidney by increasing apical lysosomal exocytosis in renal proximal tubular epithelial cells [57]. All these data indicate that lysosomal exocytosis exerts a protective function by releasing toxic heavy metals in the extracellular space.

2.3.2. Release of aggregated proteins

Most neurodegenerative diseases are characterized by the aggregation of misfolded proteins such as TAU in Alzheimer's disease or α -SYNUCLEIN in Parkinson disease. These pathogenic aggregates are released in the extracellular space and this release is proposed to initiate the neuron-to-neuron transmission leading to pathology propagation. The work of Xie et al. demonstrates that pathogenic α -SYNUCLEIN species are released from primary neurons through SNARE-dependent lysosomal exocytosis [58]. Similarly, it has been shown that TFEB regulates the exocytosis of mutant tau and this process is dependent on the lysosomal calcium channel TRPML1 [59,60]. Therefore, these data suggest that, while protecting cells through the releasing of toxic aggregates, lysosomal exocytosis plays a central role in neurodegenerative pathogenesis.

2.4. Cytotoxicity at the immunological synapse

Cytotoxic T lymphocytes (CTL) and Natural Killer cells (NK) are cytolytic effector cells of the immune system. Upon recognition and conjugation with target cells, which can be infected or tumor cells, lytic granules fuse at the plasma membrane to the site of cell–cell-contact, thus forming the cytotoxic immunological synapse. Although these granules were identified as secretory lysosomes due to their low pH and

the expression of lysosomal proteins such as LAMP1, LAMP2 and CD63 [61], these secretory lysosomes, also called “lysosome-related effector vesicles” (LREV), specifically contain proteins involved in target cell lysis (granzymes, perforin, FasL) and immunomodulation (MHC class I and II, costimulatory molecules, cytokines) (for review, [62,63]). After LREV release, the pore-forming perforin then facilitates the entry of the granzymes into the target cell cytoplasm, where they cleave a variety of targets, such as caspases, resulting in cell death. More recently, two different LREV populations were identified, based on their proteome and their release-triggering signals. The first one, light vesicles containing FasL, are released in a PKC-dependent manner while dense vesicles, including perforin and granzymes, are released in a Ca^{2+} -dependent way reminiscent of the “classical” lysosomal exocytosis [62,64–66].

3. Lysosomal exocytosis and autophagy proteins: cooperation to protect cells

Macroautophagy (hereafter referred as autophagy), is a major catabolic process which eliminates and recycles damaged proteins and organelles for homeostasis and cell survival under stress conditions. The process is initiated into the cytoplasm by the formation of a double membrane called the phagophore, through the interaction of autophagy proteins named “ATG proteins”. Briefly, the phagophore closes around the damaged material leading to an autophagosome, this autophagosome then fuses with the lysosome and the material is degraded and recycled [67].

Autophagy and lysosome pathway are two interconnected degradation processes. As organelles, lysosomes and autophagosomes (after fusion with lysosomes) are indeed primarily involved in degradation. Then, both organelles can also be involved in secretion. This case for lysosomes with lysosomal exocytosis, and it is now established that autophagosomes can be diverted from the degradation pathway to the secretion pathway under specific circumstances [68,69]. The connection between autophagy and lysosomal exocytosis is also illustrated by common key regulatory genes such as TFEB, which is a master transcription regulator of both processes [70]. In addition, recent results summarized hereafter suggest that these two pathways are more intricately than previously expected (Table 1).

First, in the case of plasma membrane damage/remodeling, various ATG proteins are required for lysosomal exocytosis [71–73]. The ATG5-ATG12-ATG16L1 complex is necessary for autophagosome formation and expansion by catalyzing microtubule-associated protein 1 light chain 3 B (LC3-I) lipidation into LC3-II [67]. In the case of plasma membrane damage due to pathogen infection, ATG16L1 and its binding partners ATG5 and ATG12 have been shown to be required for PMR in response to bacterial pore-forming toxins, through an autophagy-independent pathway [71,74]. Moreover, one of these studies also showed that ATG16L1 is required for lysosomal exocytosis as its inactivation leads to cholesterol accumulation in lysosomes [74] and an excess of cholesterol paralyzes lysosomal traffic and

Table 1

Involvement of ATG proteins in lysosomal exocytosis. In the case of PM damage/remodeling, some ATG proteins are required for lysosomal exocytosis. Conversely, inactivation of some ATG proteins can lead to increased lysosomal exocytosis. PM: Plasma membrane.

PM damage/remodeling	ATG protein	Lysosomal exocytosis	Reference
<i>Candida albicans</i>	ATG5, ATG16L1	+	Lapaquette 2022
<i>Listeria monocytogenes</i>	ATG5, ATG16L1, ATG12	+	Tan 2018
Ruffled boarder formation	ATG5, ATG7, ATG4B, LC3	+	DeSelm 2011
No	ATG5 inactivation	+++	Wang 2023
No	LC3C inactivation	+++	Verma 2023

exocytosis [75]. More recently, Lapaquette et al. demonstrated that ATG5 and ATG16L1 contribute to lysosomal exocytosis at damaged plasma membranes during *C. albicans* infection in epithelial cells [73]. Similarly, in the context of bone resorption where an extensive plasma membrane remodeling occurs, DeSelm et al. demonstrate that the autophagy proteins LC3, ATG5, ATG7, ATG4/B are involved in the targeting of lysosomes to the plasma membrane of osteoclast, thereby participating to ruffled border formation and function [73]. In both cases, ATG proteins orchestrate the release of lysosomal material in the extracellular compartment.

Conversely, loss of some ATG proteins has recently been shown to promote lysosomal exocytosis, in particular in the case of lysosomal damage [56,76]. Indeed, Deretic's group has recently demonstrated in several human cell lines that *Atg5* inactivation leads to an increase in lysosomal exocytosis due to a defective repair of lysosomal damage. Thus, in WT cells, lysosomal damage is repaired by the intervention of the ALIX protein and endosomal sorting complex required for transport (ESCRT). In *Atg5* KO cells, ALIX is sequestered in the cytoplasm by a neo-complex composed of ATG12 and ATG3, and therefore is no more able to interact with ESCRT, leading to the persistence of lysosomal damage. This persistent lysosomal damage leads to an increase of lysosomal exocytosis, without any PM damage. This effect appears to be specific to *Atg5* inactivation, as *Atg7* disruption does not give rise to the same results [76]. Similarly, loss of LC3C also stimulates lysosomal exocytosis [56]. LC3C is a paralog of LC3B and LC3A only presents in higher primates including humans. Lipidated LC3C is inserted into the autophagosome membrane during the expansion process and binds cargo receptors to the autophagosome membrane. In renal clear carcinoma cell lines expressing high level of LC3C, Verma et al. showed that loss of LC3C leads to peripheral positioning and exocytosis of the lysosomes. These results indicate that both lysosome localization and exocytosis are dependent of LC3C in an autophagy-independent way [56].

Therefore, in the case of plasma membrane damage due to pathogen infection or plasma membrane remodeling due to ruffled border formation in osteoclasts, various ATG proteins are needed for lysosomal exocytosis [72–74]. Conversely, loss of some ATG proteins such as ATG5 and LC3C triggers lysosomal exocytosis [56,76], which is related to the presence of damaged lysosomes, at least in the case of ATG5 [76]. These data suggest that in both situations, ATG proteins exert a protective role, either to promote PMR or to avoid lysosomal disrepair. Taken together, these results indicate that a subset of ATG proteins are involved in lysosomal exocytosis, likely through non-canonical autophagy-related processes. Further studies will be required to decipher the precise mechanism(s) by which these autophagy proteins modulate this process according to the context i.e. PM damage/remodeling or lysosome

damage.

4. 4- Lysosomal exocytosis in cancer cells

Table 2 presents a synthesis of main available data about the current knowledge of lysosomal exocytosis in cancer. Lysosomal exocytosis plays a key role in various cancers, and its activation is correlated to several protumoral aspects: drug resistance, increased proliferation, invasion and migration [20,77–85]. The different elements released during lysosomal exocytosis are involved in various protumoral mechanisms summarized below (Fig. 3).

4.1. Proton release: tumor microenvironment acidification

As lysosomes are major intracellular proton repositories, lysosomal exocytosis results in local extracellular acidosis [86] which has been evidenced in specialized compartments like the resorption lacunae in bone [87], the “lysosomal synapse” of macrophages for extracellular degradation of aggregated low-density lipoproteins [88], or the B-cell synapse to promote the extraction of the immobilized Ag [89]. This acidosis can modify cancer cell expression to promote tumor growth and immune escape. For example, an acidic pH medium stimulates vascular endothelial growth factor (VEGF) production in human glioblastoma cells [90]. Similarly, low pH significantly increases IFN- γ -induced programmed death-ligand 1 (PD-L1) expression in cancer cells, which can result in T cell exhaustion and immune escape [91]. Acidic stress was also shown to select the emergence of aggressive pancreatic cancer clones through genetic reprogramming that promotes proliferation, migration, invasion, autophagy and epithelial-to-mesenchymal transition and results in cancer cells with enhanced metastatic potential [92]. This was also observed *in vivo* with acid-adapted breast cancer cells exhibiting an enhanced tumor growth compared to their WT counterpart [93]. In addition to its action on cancer cells, acidosis can also regulate both innate and adaptive immune responses [94]. Dendritic cells (DCs) are specialized antigen-presenting cells with a unique ability to prime naive T cells to induce an adaptive immune response. Extracellular acidosis was shown to trigger the phenotypic maturation of human DCs [95,96] and to improve uptake of antigens and MHC class I-restricted presentation in mouse DCs [97]. Conversely, extracellular acidosis was shown to alter macrophage activation *in vitro* and to induce a tumor-promoting phenotype [98]. Lowering the environmental pH to values that characterize tumors (pH 6–6.5) induces an anergy in human and mouse tumor-specific CD8⁺ T cells, with an impairment of cytotoxic activity, an inhibition of cytokine secretion and a reduced activation of proteins involved in signal transduction following T-cell receptor activation [99]. Low pH also markedly inhibits the cytotoxic response of NK

Table 2
Effect of lysosomal exocytosis observed in different types of cancer.

Type of cancer	Model	Drug resistance	Invasion Migration	Mechanism	References
Renal cell carcinoma	Sunitinib-resistant cell line with basal high level of LE	+	+	Sunitinib efflux and cathepsin release	Li et al., 2021
Hepatocarcinoma (HCC)	HCC cells with high level of LE	ND	+	TRPML1-dependent	Lyu et al., 2020
Melanoma	Primary melanoma, UVA induced LE	ND	+	Cathepsin-dependent	Eding et al., 2015
Neuroblastoma	Chemotherapy-resistant neuroblastoma cell line	+	ND	Sensitization to doxorubicin by LE inhibition	Ridinger et al., 2018
Non-small cell lung cancer	Gefitinib-resistant non-small cell lung cancer cell line with high LE level	+	ND	TRMPL3-dependent	Kim et al., 2022
Pleomorph rhabdomyosarcoma/ Sarcomas	Neu1 ^{+/-} /Arf ^{-/-} mouse model and primary cells with high level of LE	+	ND	Chemoresistance reversed by chemical or genetical LE inhibition	Machado et al., 2015 & 2022
Oral squamous carcinoma	Gefitinib-resistant cell line	+	ND	ABCA2-dependent	Kim et al., 2023
Glioma	Glioma cell lines	ND	+	Rab27A-dependent Cathepsin D-dependent	Liu et al., 2012
Ovarian	Ovarian cancer cells	+	ND	Cisplatin resistance TRPML1-dependant	Kim et al., 2024

ND: not described. LE: lysosomal exocytosis.

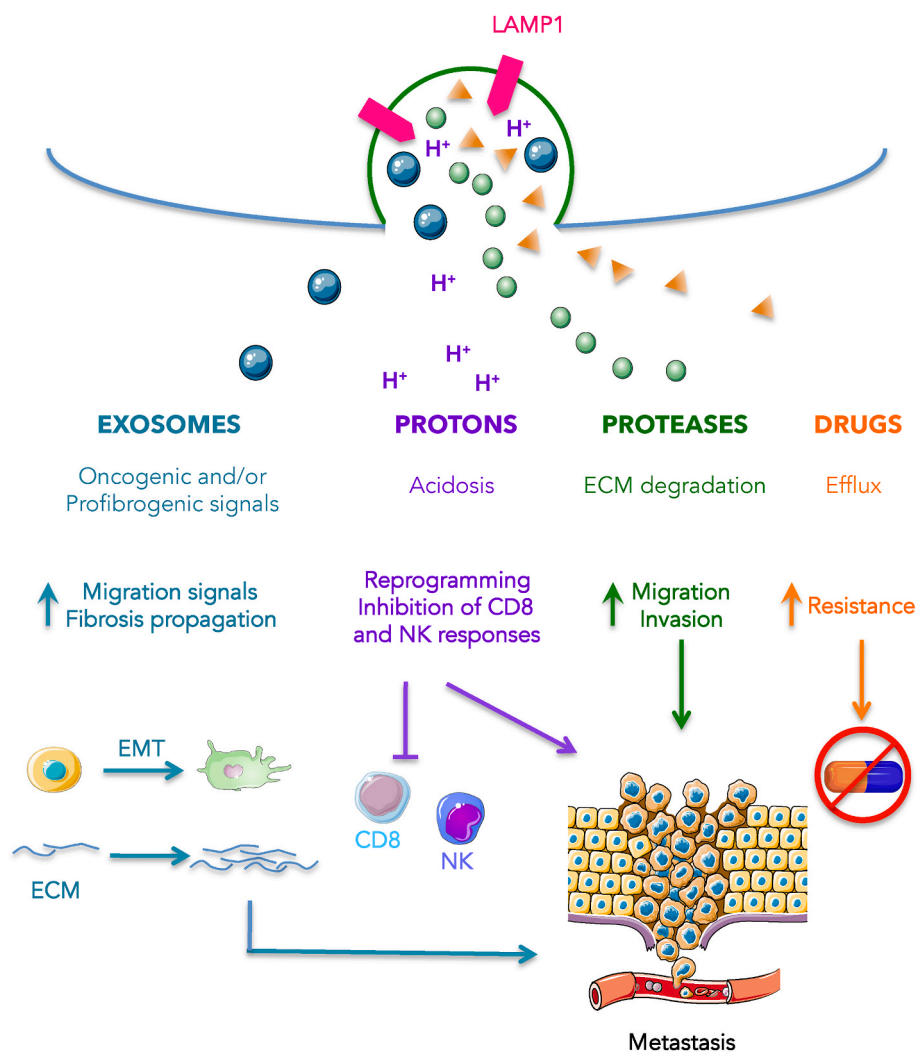


Fig. 3. Roles of lysosomal exocytosis in cancer progression.

All the elements released during lysosomal exocytosis (exosomes, protons and proteases) are involved in tumor progression by facilitating migration/invasion. Acidosis generated by protons secretion blunts cytotoxic immune responses. Proteases and exosomes are involved in extracellular matrix (ECM) remodeling and favor the creation of a fibrotic microenvironment. Protease release is also involved in radioresistance. Lysosomotropic chemotherapeutic drugs are released during lysosomal exocytosis, inducing treatment resistance.

cells against tumor cells through the reduction of perforin/granzyme-containing granule release and cytokine secretion [100]. Taken together, these data indicate extracellular acidosis promotes tumor development, metastatic potential of cancer cells and inhibits immune responses.

4.2. Protease release

4.2.1. Migration/invasion associated with extracellular matrix degradation

Among the lysosomal proteases, cathepsins play a major role in extracellular matrix degradation and tumor cell invasion. These enzymes remain functionally active outside of the lysosome and are divided in three protease families: serine proteases (cathepsins A and G), aspartic proteases (cathepsin D and E) and cysteine cathepsins (cathepsins B, C, F, H, K, L, O, S, V, X, and W) [101]. Accumulation of anticancer drugs in lysosomes leads to fusion of lysosomes with the plasma membrane and this drug-induced lysosomal exocytosis results in the secretion of lysosomal proteases such as cathepsin D [102]. Cathepsin D was shown to stimulate proliferation, survival, motility, invasion and angiogenesis in epithelial breast cancer cell lines [103]. In addition, a decreased migration capacity can be obtained in glioma cell lines, by inhibiting either cathepsin D or lysosomal exocytosis [84]. Role

of cysteine cathepsins released in the microenvironment have also been demonstrated in tumor growth, migration, invasion, angiogenesis and metastasis, through their downregulation or inactivation in cell lines and in human cancer models in transgenic mice [104]. In particular, upregulated secretion of cathepsin L is observed in a wide range of human cancers and plays a major role in cancer cell dissemination through extracellular matrix degradation [105]. The gene TMEM106B, which has been identified as a driver of lung cancer metastasis, induces lysosomal exocytosis and thereby the release of cysteine cathepsins that are required for the TMEM106B-mediated metastatic spread of cancer cells [106]. The protumoral roles of extracellular cathepsins are at least in part mediated through extracellular matrix degradation which in turn facilitates tumor dissemination, shedding of membrane proteins potentially interfering with protumoral transduction pathways [101].

4.2.2. Radioresistance

With chemoresistance, cancer radioresistance is topic of interest to improve patient care. Several experimental arguments suggest a potential relationship between protease release through lysosomal exocytosis and radioresistance. Indeed, radiation can induce cathepsin protease production and cathepsins have been shown to play a role in radiation resistance in glioma and breast cancer cells [107,108].

Similarly, TFEB is upregulated after radiation, and knockdown of TFEB enhances radiosensitivity [109]. Finally, the direct role of lysosomal exocytosis in radiation resistance was recently demonstrated by Wu et al. [110]. These authors showed that lysosomal exocytosis was increased in human breast adenocarcinoma cells surviving to ionizing radiation (IR). Lysosomal exocytosis was due to enhanced activation of ARL8B, a key regulator of lysosome positioning to the cell periphery. ARL8B-dependent lysosomal exocytosis was shown to play a major role in the enhanced invasiveness of cells that survive IR through an increased degradation of the extracellular matrix. Finally, ARL8B was shown to be essential for the increased tumor growth and metastasis of the invasive cancer cells surviving IR [110].

4.3. Release of lysomotropic chemotherapeutics: treatment resistance

Drug resistance represents an important clinical challenge in cancer treatment and lysosomes contribute to this phenomenon. Indeed, it is known that some hydrophobic weak base chemotherapeutics and tyrosine kinase inhibitors such as doxorubicin and sunitinib respectively, accumulate in lysosomes [111]. This drug accumulation is by itself a sufficient signal to trigger lysosomal exocytosis [102,111]. Although sunitinib is an effective treatment in renal cell carcinoma, most patients develop resistance to this tyrosine kinase inhibitor. Using a model of sunitinib-resistant cell line, Li et al. have shown that once the resistance is acquired, sunitinib promotes resistance via lysosomal exocytosis. Indeed, they observed that sunitinib enhances the interaction between the endoplasmic reticulum (ER) protein E-SYT 1 and the lysosomal protein SYT VII. This interaction promotes ER fragmentation and calcium release, stimulating lysosomal exocytosis and reinforcing sunitinib resistance by pumping it out from the cytoplasm [77]. Similarly, it has been shown that lysosomal exocytosis is higher in the gefitinib-resistant non-small cell lung cancer cell line than in the gefitinib-sensitive cell line in a TRMPL3 dependent way [81]. Soft tissue sarcomas are some of the most aggressive and untreatable tumors. Machado et al. explored the role of lysosomal exocytosis in the development and the aggressiveness of these tumors using a mouse model of *Neu1* haploinsufficiency (*Neu1*^{+/-}/*Arf*^{-/-}) with increased lysosomal exocytosis. In this model, the authors showed that primary sarcoma cells exhibit increased lysosomal exocytosis, which promotes the efflux of lysosomal doxorubicin, providing a mechanism for chemoresistance. They confirmed that lysosomal exocytosis was the key mechanism responsible of this chemoresistance demonstrating that its chemical (verapamil) or genetical (shLamp1 and/or shMyh11) inhibition reversed invasiveness and chemoresistance. Thus, excessive lysosomal exocytosis subsequent to low NEU1 levels may be a major process leading sarcomas to acquire resistance to lysomotropic chemotherapeutics *in vivo* [20]. These data suggest that resistance to treatment is promoted by the efflux of lysomotropic drugs, which together with hydrolase secretion, promotes survival, invasion and dissemination of cancer cells [102].

4.4. Tumor exosome release: propagation of oncogenic/profibrotic signals

Exosomes are extracellular vesicles originating from late endosomes called multivesicular bodies (MVBs) by inward budding of the membrane. MVBs can fuse with the plasma membrane to release exosomes but can also fuse with lysosomes where exosomes can be either degraded, or protected from degradation and released by lysosomal exocytosis [20,112]. Exosomes contain macromolecules such as proteins, lipids, RNAs or even DNA, which are responsible for their functions. It is currently unknown whether lysosomal exocytosis facilitates docking of MVBs at the plasma membrane or if fusion of plasma membrane-docked lysosomes with MVBs occurs before lysosomal exocytosis [33]. Whatever the mechanism, lysosomal exocytosis and exosome release appear to be frequently associated, not only in cancer situations. This is for example the case in preeclamptic placentae where ceramide accumulation was shown to be responsible for both increased

TFEB-induced lysosomal exocytosis and exosome release [113]. This was also shown during adipogenesis, where adipogenic stimuli triggers TRPML1-regulated exosomal release by mediating lysosomal exocytosis [112]. Finally, it was recently demonstrated that *Neu1*^{-/-} fibroblasts, which exhibit excessive lysosomal exocytosis, have features of myofibroblasts and exocytose large amount of exosomes [33]. Therefore, upregulation of genes stimulating lysosomal exocytosis (TFEB, TRPML1) or inactivation of lysosomal exocytosis negative regulator (*Neu1*) increase exosome release although the precise mechanism is still to be defined. In the case of *Neu1*^{-/-} fibroblasts, exosomes which are loaded with TGF- β and wntless-related integration site (WNT)/ β -catenin signaling molecules, are able to propagate profibrotic signals to neighboring cells and educate them to acquire myofibroblasts properties [33]. Similarly, *Neu1*^{+/-} rhabdomyosarcoma, which presents increased lysosomal exocytosis, promotes a fibrotic microenvironment [82]. Finally, in the context of liver fibrogenesis, the highly expressed P2X4 purinergic receptor has been implicated in the profibrogenic properties of hepatic myofibroblasts. This effect appears to be, at least in part, related to the P2X4-dependent regulation of lysosomal exocytosis [114]. In addition to the propagation of profibrotic signals, exosomes released by lysosomal exocytosis may condition neighboring cells to become migratory. This was demonstrated by migration stimulation of a sarcoma cell line with WT NEU1 activity in the presence of exosomes from a sarcoma cell line with low NEU1 activity [20]. Finally, autophagy machinery is also connected to exosome release/degradation [115], suggesting another potential cooperation between lysosomal exocytosis and ATG proteins in this context. Taken together, these data indicate that lysosomal exocytosis can participate to the propagation of oncogenic and/or profibrotic signals through exosome release, thereby contributing to microenvironment modification. For further details on exosomes and tumoral microenvironment, see reviews [116,117].

4.5. Cell reprogramming

Some studies also described that lysosomal exocytosis or its consequence, i.e. acidosis, can be associated with transcriptional and metabolic reprogramming. The work of Verma et al. recently compared renal cancer cells exhibiting high and low lysosomal exocytosis at the transcriptomic and metabolomic levels [56]. Renal cancer cells with high lysosomal exocytosis activity exhibit an altered expression of Zinc (Zn)-related genes such as Zn finger transcription factors and leads to Zn-dependent epigenetic reprogramming [56]. In addition, the authors showed that lysosomal exocytosis activation results in metabolic reprogramming with a significant decrease in most amino acids. Finally, these renal cancer cells with high lysosomal exocytosis activity showed increased tumor-initiating properties and form aggressive tumors *in vivo* [56]. Transcriptome rewiring was also observed for pancreatic cancer cells submitted to prolonged acidosis with a genetic reprogramming promoting proliferation, migration, invasion, autophagy and epithelial-to-mesenchymal transition. This pro-metastatic phenotype was conserved following the restoration of a physiological external pH, suggesting the selection of aggressive cancer clones [92].

Taken together, these data indicate that lysosomal exocytosis could have more profound effects on cell fate and behavior than previously expected.

5. 5- Conclusions and perspectives

The main function of lysosomes is degradation. However, in some circumstances which begin to be elucidated, lysosomal content is released extracellularly. Lysosomal exocytosis exerts crucial roles in various normal physiological processes such as bone resorption or antigen presentation in which protons and/or proteases participate to the function. Interestingly, the cells also appear to use lysosomal exocytosis as an emergency response to repair plasma membrane damage, to promote clearance of toxic products such as heavy metals or pathogenic

aggregates, or to release extracellularly lysosomal content in the case of lysosomal membrane disrepair.

From a mechanistic point of view, TFEB and TRPML1 are key regulators of this complex cascade of events involving numerous actors which discovery is probably not yet completed. For instance, some proteins from the autophagic machinery were first shown to contribute to lysosomal exocytosis in the case of bone resorption [54] and these findings were recently extended to the infection process by pathogens such as *Candida albicans* and *Listeria monocytogenes* [56,57]. Further studies will be required to determine in which situations ATG proteins are involved in this process and what are the signals which trigger this participation.

In the context of cancer, lysosomal exocytosis is receiving an increasing interest as the different elements released in the tumor microenvironment i.e. proteases, exosomes, and protons, are able to stimulate the metastatic behavior of cancer cells. This stimulation includes enhancement of tumor cell migration and invasion through extracellular matrix remodeling, promotion of a fibrotic protumoral soil, selection of aggressive cancer clones and inhibition of the anti-tumor immune responses. Finally, lysosomal exocytosis contributes to treatment resistance through lysosomotropic drug efflux and protease secretion. These data suggest that lysosomal exocytosis is indeed an early therapeutic target in cancer. In this context, the inhibition of TRPML1 function appears to be an attractive approach, at the crossroads between autophagy modulation and lysosomal exocytosis inhibition [118]. TRPML1 inhibition has been performed in myocardial ischemia and in some cancers such as melanoma or hepatocarcinoma, where it decreases tumor metastatic potential [78,118,119]. Other strategies have been explored to inhibit the effect of some lysosomal proteins released during exocytosis. Due to its major role in cancer cells dissemination, cathepsin L appears as a particularly attractive target [120]. In this context, KGP94, a cathepsin L inhibitor, has been shown to decrease metastatic potential in breast and cancer cell lines under normal or acidic/hypoxic microenvironment [105]. Although these results are encouraging, none of these molecules have still achieved the expected clinical results. Another promising possibility is to act upstream, before the engagement of the lysosomal exocytosis process, by inducing major and non-repairable lysosomal membrane damages to trigger lysosomal-dependent cancer cell death [9,121].

In conclusion, studies to date show that lysosomal exocytosis is a cellular process involved in many physiological functions and that it can be used in emergency situations to protect the cell. This process can also be hijacked by tumor cells to their own advantage. Because of this dual role, a better understanding of the actors involved in this mechanism will be necessary in order to develop effective anti-tumoral strategies based on its inhibition.

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CRedit authorship contribution statement

Marie-Charlotte Trojani: Writing – review & editing, Writing – original draft, Investigation. **Sabine Santucci-Darmanin:** Writing – review & editing. **Véronique Breuil:** Writing – review & editing, Funding acquisition. **Georges F. Carle:** Writing – review & editing, Writing – original draft. **Valérie Pierrefite-Carle:** Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

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References

- [1] C. De Duve, B.C. Pressman, R. Gianetto, R. Wattiaux, F. Appelmans, Tissue fractionation studies. 6. Intracellular distribution patterns of enzymes in rat-liver tissue, *Biochem. J.* 60 (4) (1955 Aug) 604–617.
- [2] P. Saftig, A. Haas, Turn up the lysosome, *Nat. Cell Biol.* 18 (10) (2016 Sep 28) 1025–1027.
- [3] S. Piao, R.K. Amaravadi, Targeting the lysosome in cancer, *Ann. N. Y. Acad. Sci.* 1371 (1) (2016 May) 45–54.
- [4] J. Kos, A. Mitrović, M. Perišić Nanut, A. Pišlar, Lysosomal peptidases-intriguing roles in cancer progression and neurodegeneration, *FEBS Open Bio* 12 (4) (2022 Apr) 708–738.
- [5] P.C. Trivedi, J.J. Bartlett, T. Pulinilkunnil, Lysosomal Biology and function: Modern view of cellular Debris Bin, *Cells* 9 (5) (2020 May 4) 1131.
- [6] R.D. Bagshaw, D.J. Mahuran, J.W. Callahan, A proteomic Analysis of lysosomal integral membrane proteins Reveals the Diverse Composition of the organelle, *Mol. Cell. Proteomics* 4 (2) (2005 Feb 1) 133–143.
- [7] C. Yang, X. Wang, Lysosome biogenesis: regulation and functions, *J. Cell Biol.* 220 (6) (2021 Jun 7) e202102001.
- [8] G. Vaes, ON the mechanisms OF BONE RESORPTION, *J. Cell Biol.* 39 (3) (1968 Dec 1) 676–697.
- [9] E.R. Machado, I. Annunziata, D. van de Vlekkert, G.C. Grosveld, A. d’Azzo, Lysosomes and cancer progression: a Malignant Liaison, *Front. Cell Dev. Biol.* 9 (2021) 642494.
- [10] J.S. Bonifacino, J. Neefjes, Moving and positioning the endolysosomal system, *Curr. Opin. Cell Biol.* 47 (2017 Aug) 1–8.
- [11] B. Tancini, S. Buratta, F. Delo, K. Sagini, E. Chiaradia, R.M. Pellegrino, et al., Lysosomal exocytosis: the extracellular role of an intracellular organelle, *Membranes* 10 (12) (2020 Dec 9) 406.
- [12] N. Hirokawa, Y. Noda, Y. Tanaka, S. Niwa, Kinesin superfamily motor proteins and intracellular transport, *Nat. Rev. Mol. Cell Biol.* 10 (10) (2009 Oct) 682–696.
- [13] B. Cabukusta, J. Neefjes, Mechanisms of lysosomal positioning and movement, *Traffic* 19 (10) (2018) 761–769.
- [14] C. Rosa-Ferreira, S. Munro, Arl8 and SKIP act together to link lysosomes to kinesin-1, *Dev. Cell* 21 (6) (2011 Dec 13) 1171–1178.
- [15] J. Pu, C. Schindler, R. Jia, M. Jarnik, P. Backlund, J.S. Bonifacino, BORC, a multisubunit complex that regulates lysosome positioning, *Dev. Cell* 33 (2) (2015 Apr 20) 176–188.
- [16] R. De Pace, R. Maroofian, A. Paimboeuf, M. Zamani, M.S. Zaki, S. Sadeghian, et al., Biallelic BORCS8 variants cause an infantile-onset neurodegenerative disorder with altered lysosome dynamics, *Brain J Neurol* (2023 Dec 21) awad427.
- [17] C.M. Guardia, G.G. Farias, R. Jia, J. Pu, J.S. Bonifacino, BORC functions upstream of kinesins 1 and 3 to Coordinate Regional movement of lysosomes along different microtubule Tracks, *Cell Rep.* 17 (8) (2016 Nov 15) 1950–1961.
- [18] E.L. Eskelinen, Roles of LAMP-1 and LAMP-2 in lysosome biogenesis and autophagy, *Mol Aspects Med* 27 (5–6) (2006 Oct) 495–502.
- [19] E.L. Eskelinen, Y. Tanaka, P. Saftig, At the acidic edge: emerging functions for lysosomal membrane proteins, *Trends Cell Biol.* 13 (3) (2003 Mar) 137–145.
- [20] E. Machado, S. White-Gilbertson, D. van de Vlekkert, L. Janke, S. Moshiah, Y. Campos, et al., Regulated lysosomal exocytosis mediates cancer progression, *Sci. Adv.* 1 (11) (2015 Dec 18) e1500603.
- [21] M. Encarnação, L. Espada, C. Escreveante, D. Mateus, J. Ramalho, X. Michelet, et al., A Rab3a-dependent complex essential for lysosome positioning and plasma membrane repair, *J. Cell Biol.* 213 (6) (2016 Jun 20) 631–640.
- [22] C. Escreveante, L. Bento-Lopes, J.S. Ramalho, D.C. Barral, Rab11 is required for lysosome exocytosis through the interaction with Rab3a, Sec15 and GRAB, *J. Cell Sci.* 134 (11) (2021 Jun 1) jcs246694.
- [23] H. Lachuer, L. Le, S. Lévêque-Fort, B. Goud, K. Schauer, Spatial organization of lysosomal exocytosis relies on membrane tension gradients, *Proc Natl Acad Sci* 120 (8) (2023 Feb 21) e2207425120.
- [24] J. Lin, A.P. McCann, N. Sereesongsang, J.M. Burden, A.A. Alsa’d, R.E. Burden, et al., USP17 is required for peripheral trafficking of lysosomes, *EMBO Rep.* 23 (4) (2022 Jan 26) e51932.
- [25] B.S. Kilpatrick, E. Yates, C. Grimm, A.H. Schapira, S. Patel, Endo-lysosomal TRP mucolipin-1 channels trigger global ER Ca²⁺ release and Ca²⁺ influx, *J. Cell Sci.* 129 (20) (2016 Oct 15) 3859–3867.
- [26] D.L. Medina, A. Fraldi, V. Bouche, F. Annunziata, G. Mansueto, C. Spampinato, et al., Transcriptional activation of lysosomal exocytosis promotes cellular clearance, *Dev. Cell* 21 (3) (2011 Sep 13) 421–430.
- [27] S. Di Paola, A. Scotto-Rosato, D.L. Medina, TRPML1: the Ca(2+)retaker of the lysosome, *Cell Calcium* 69 (2018 Jan) 112–121.
- [28] D.L. Medina, A. Fraldi, V. Bouche, F. Annunziata, G. Mansueto, C. Spampinato, et al., Transcriptional activation of lysosomal exocytosis promotes cellular clearance, *Dev. Cell* 21 (3) (2011 Sep 13) 421–430.
- [29] S. Di Paola, D.L. Medina, TRPML1-/TFEB-Dependent regulation of lysosomal exocytosis, *Methods Mol Biol Clifton NJ* 1925 (2019) 143–144.
- [30] W. Wang, X. Zhang, Q. Gao, H. Xu, TRPML1: an ion channel in the lysosome, *Handb. Exp. Pharmacol.* 222 (2014) 631–645.
- [31] S.K. Rao, C. Huynh, V. Proux-Gillardeaux, T. Galli, N.W. Andrews, Identification of SNAREs involved in synaptotagmin VII-regulated lysosomal exocytosis, *J. Biol. Chem.* 279 (19) (2004 May) 20471–20479.
- [32] N.W. Andrews, Detection of lysosomal exocytosis by surface exposure of Lamp1 luminal Epitopes [cited 2023 Sep 19], in: K. Öllinger, H. Appelqvist (Eds.),

- Lysosomes: Methods and Protocols [Internet], *Methods in Molecular Biology*, New York, NY: Springer, 2017, pp. 205–211, https://doi.org/10.1007/978-1-4939-6934-0_13.
- [33] D. van de Vlekkert, J. Demmers, X.X. Nguyen, Y. Campos, E. Machado, I. Annunziata, et al., Excessive exosome release is the pathogenic pathway linking a lysosomal deficiency to generalized fibrosis, *Sci. Adv.* 5 (7) (2019 Jul) eaav3270.
- [34] A. Gorelik, K. Illes, M.T. Mazhab-Jafari, B. Nagar, Structure of the immunoregulatory sialidase NEU1, *Sci. Adv.* 9 (20) (2023 May 19) eadf8169.
- [35] G. Yogalingam, E.J. Bonten, D. van de Vlekkert, H. Hu, S. Moshiah, S.A. Connell, et al., Neuraminidase 1 is a negative regulator of lysosomal exocytosis, *Dev. Cell* 15 (1) (2008 Jul) 74–86.
- [36] E.J. Bonten, I. Annunziata, A. d’Azzo, Lysosomal multienzyme complex: pros and cons of working together, *Cell Mol Life Sci CMLS* 71 (11) (2014 Jun) 2017–2032.
- [37] A. Khan, C. Sergi, Sialidosis: a review of Morphology and molecular Biology of a Rare Pediatric disorder, *Diagnosics* 8 (2) (2018 Apr 25) 29.
- [38] D. Roy, D.R. Liston, V.J. Idone, A. Di, D.J. Nelson, C. Pujol, et al., A process for controlling intracellular bacterial infections induced by membrane injury, *Science* 304 (5676) (2004 Jun 4) 1515–1518.
- [39] D. Keefe, L. Shi, S. Feske, R. Massol, F. Navarro, T. Kirchhausen, et al., Perforin triggers a plasma membrane-repair response that facilitates CTL induction of apoptosis, *Immunity* 23 (3) (2005 Sep) 249–262.
- [40] C.L. Forestier, C. Machu, C. Loussert, P. Pescher, G.F. Späth, Imaging host cell-Leishmania interaction dynamics implicates parasite motility, lysosome recruitment, and host cell wounding in the infection process, *Cell Host Microbe* 9 (4) (2011 Apr 21) 319–330.
- [41] C. Tam, V. Idone, C. Devlin, M.C. Fernandes, A. Flannery, X. He, et al., Exocytosis of acid sphingomyelinase by wounded cells promotes endocytosis and plasma membrane repair, *J. Cell Biol.* 189 (6) (2010 Jun 14) 1027–1038.
- [42] T. Castro-Gomes, M. Corrotte, C. Tam, N.W. Andrews, Plasma membrane repair is regulated extracellularly by proteases released from lysosomes, *PLoS One* 11 (3) (2016) e0152583.
- [43] L.J. Raggatt, N.C. Partridge, Cellular and molecular mechanisms of bone remodeling, *J. Biol. Chem.* 285 (33) (2010 Aug) 25103–25108.
- [44] N.S. Soysa, N. Alles, Osteoclast function and bone-resorbing activity: an overview, *Biochem. Biophys. Res. Commun.* 476 (3) (2016 Jul 29) 115–120.
- [45] A. Elson, A. Anuj, M. Barnea-Zohar, N. Reuven, The origins and formation of bone-resorbing osteoclasts, *Bone* 164 (2022 Nov) 116538.
- [46] R. Baron, L. Neff, W. Brown, P.J. Courtot, D. Louvard, M.G. Farquhar, Polarized secretion of lysosomal enzymes: co-distribution of cation-independent mannose-6-phosphate receptors and lysosomal enzymes along the osteoclast exocytic pathway, *J. Cell Biol.* 106 (6) (1988 Jun) 1863–1872.
- [47] H. Zhao, Y. Ito, J. Chappel, N.W. Andrews, S.L. Teitelbaum, F.P. Ross, Synaptotagmin VII regulates bone remodeling by modulating osteoclast and osteoblast secretion, *Dev. Cell* 14 (6) (2008 Jun) 914–925.
- [48] Y. Miyatake, T. Yamano, R. Hanayama, Myoferlin-mediated lysosomal exocytosis regulates cytotoxicity by phagocytes, *J. Immunol. Baltim Md* 201 (10) (1950. 2018 Nov 15) 3051–3057.
- [49] M.I. Yuseff, A. Reversat, D. Lankar, J. Diaz, I. Fanget, P. Pierobon, et al., Polarized secretion of lysosomes at the B cell synapse couples antigen extraction to processing and presentation, *Immunity* 35 (3) (2011 Sep 23) 361–374.
- [50] Maeda FY, van Haaren JJ, Langley DB, Christ D, Andrews NW, Song W. Surface-associated antigen induces permeabilization of primary mouse B-cells and lysosome exocytosis facilitating antigen uptake and presentation to T-cells. *Elife*. 10:e66984.
- [51] F.D. Batista, M.S. Neuberger, B cells extract and present immobilized antigen: implications for affinity discrimination, *EMBO J.* 19 (4) (2000 Feb 15) 513–520.
- [52] C. Nugues, D. Rajamanoharan, R.D. Burgoyne, L.P. Haynes, N. Helassa, Lysosome exocytosis is required for mitosis in mammalian cells, *Biochem. Biophys. Res. Commun.* 626 (2022 Oct 20) 211–219.
- [53] B. Fernández, P. Olmedo, F. Gil, E. Fdez, Y. Naaldijk, P. Rivero-Ríos, et al., Iron-induced cytotoxicity mediated by endolysosomal TRPML1 channels is reverted by TFEB, *Cell Death Dis.* 13 (12) (2022 Dec 16) 1047.
- [54] E.V. Polishchuk, M. Concilli, S. Iacobacci, G. Chesì, N. Pastore, P. Piccolo, et al., Wilson disease protein ATP7B utilizes lysosomal exocytosis to maintain copper homeostasis, *Dev. Cell* 29 (6) (2014 Jun 23) 686–700.
- [55] K. Peña, J. Coblenz, K. Kiselyov, Brief exposure to copper activates lysosomal exocytosis, *Cell Calcium* 57 (4) (2015 Apr 1) 257–262.
- [56] R. Verma, P. Aggarwal, M.E. Bischoff, J. Reigle, D. Secic, C. Wetzel, et al., Microtubule-associated protein MAP1LC3C regulates lysosomal exocytosis and induces zinc reprogramming in renal cancer cells, *J. Biol. Chem.* 299 (5) (2023 May) 104663.
- [57] D. Zhong, R. Wang, H. Zhang, M. Wang, X. Zhang, H. Chen, Induction of lysosomal exocytosis and biogenesis via TRPML1 activation for the treatment of uranium-induced nephrotoxicity, *Nat. Commun.* 14 (1) (2023 Jul 6) 3997.
- [58] Y.X. Xie, N.N. Naseri, J. Fels, P. Kharel, Y. Na, D. Lane, et al., Lysosomal exocytosis releases pathogenic α -synuclein species from neurons in synucleinopathy models, *Nat. Commun.* 13 (1) (2022 Aug 22) 4918.
- [59] Y. Tanaka, K. Yamada, K. Satake, I. Nishida, M. Heuberger, T. Kuwahara, et al., Seeding activity-based Detection Uncovers the different release mechanisms of Seed-Competent tau versus Inert tau via lysosomal exocytosis, *Front Neurosci* [Internet] (2019) [cited 2024 Feb 7];13. Available from: <https://www.frontiersin.org/journals/neuroscience/articles/10.3389/fnins.2019.01258>.
- [60] Y. Xu, S. Du, J.A. Marsh, K. Horie, C. Sato, A. Ballabio, et al., TFEB regulates lysosomal exocytosis of tau and its loss of function exacerbates tau pathology and spreading, *Mol Psychiatry* 26 (10) (2021 Oct) 5925–5939.
- [61] P.J. Peters, J. Borst, V. Oorschot, M. Fukuda, O. Krähenbühl, J. Tschopp, et al., Cytotoxic T lymphocyte granules are secretory lysosomes, containing both perforin and granzymes, *J. Exp. Med.* 173 (5) (1991 May 1) 1099–1109.
- [62] M. Lettau, O. Janssen, Intra- and extracellular effector vesicles from human T and NK cells: same-same, but different? *Front. Immunol.* 12 (2021) 804895.
- [63] E.J. Blott, G.M. Griffiths, Secretory lysosomes, *Nat. Rev. Mol. Cell Biol.* 3 (2) (2002 Feb) 122–131.
- [64] H. Schmidt, C. Gelhaus, M. Nebendahl, M. Leippe, O. Janssen, Proteomic profiling of secretory granules of different T cell subpopulations, *Cell Commun. Signal.* 7 (1) (2009 Feb 26) A85.
- [65] H. Schmidt, C. Gelhaus, M. Nebendahl, M. Lettau, R. Lucius, M. Leippe, et al., Effector granules in human T lymphocytes: the luminal proteome of secretory lysosomes from human T cells, *Cell Commun Signal CCS* 9 (2011 Jan 21) 4.
- [66] M. Lettau, D. Kabelitz, O. Janssen, Lysosome-related effector vesicles in T lymphocytes and NK cells, *Scand. J. Immunol.* 82 (3) (2015) 235–243.
- [67] L. Galluzzi, E.H. Baehrecke, A. Ballabio, P. Boya, J.M. Bravo-San Pedro, F. Cecconi, et al., Molecular definitions of autophagy and related processes, *EMBO J.* 36 (13) (2017 Jul 3) 1811–1836.
- [68] J. New, S.M. Thomas, Autophagy-dependent secretion: mechanism, factors secreted, and disease implications, *Autophagy* 15 (10) (2019 Apr 14) 1682–1693.
- [69] M. Ponpuak, M. Mandell, T. Kimura, S. Chauhan, C. Cleary, V. Deretic, Secretory autophagy, *Curr. Opin. Cell Biol.* 35 (2015 Aug) 106–116.
- [70] R. Song, H. Lei, L. Feng, W. Cheng, Y. Li, L.L. Yao, et al., TFEB insufficiency promotes cardiac hypertrophy by blocking autophagic degradation of GATA4, *J. Biol. Chem.* 297 (4) (2021 Oct) 101189.
- [71] J.M.J. Tan, N. Mellouk, J.H. Brumell, An autophagy-independent role for ATG16L1: promoting lysosome-mediated plasma membrane repair, *Autophagy* 15 (5) (2019 May) 932–933.
- [72] C.J. DeSelm, B.C. Miller, W. Zou, W.L. Beatty, H. van Meel, Y. Takahata, et al., Autophagy proteins regulate the secretory component of osteoclastic bone resorption, *Dev. Cell* 21 (5) (2011 Nov 15) 966–974.
- [73] Lapaquette P, Ducreux A, Basmacyian L, Paradis T, Bon F, Bataille A, et al. Membrane protective role of autophagic machinery during infection of epithelial cells by *Candida albicans*. *Gut Microb.* 14(1):2004798.
- [74] J.M.J. Tan, N. Mellouk, S.E. Osborne, D.A. Ammendolia, D.N. Dyer, R. Li, et al., An ATG16L1-dependent pathway promotes plasma membrane repair and limits *Listeria monocytogenes* cell-to-cell spread, *Nat Microbiol* 3 (12) (2018 Dec) 1472–1485.
- [75] J. Xu, K.A. Toops, F. Diaz, J.M. Carvajal-Gonzalez, D. Gravotta, F. Mazzoni, et al., Mechanism of polarized lysosome exocytosis in epithelial cells, *J. Cell Sci.* 125 (24) (2012 Dec 15) 5937–5943.
- [76] F. Wang, R. Peters, J. Jia, M. Mudd, M. Salemi, L. Allers, et al., ATG5 provides host protection acting as a switch in the atg5ylation cascade between autophagy and secretion, *Dev. Cell* 58 (10) (2023 May 22) 866–884.e8.
- [77] L. Li, S. Zhao, Z. Liu, N. Zhang, S. Pang, J. Liu, et al., Sunitinib treatment promotes metastasis of drug-resistant renal cell carcinoma via TFE3 signaling pathway, *Cell Death Dis.* 12 (2) (2021 Feb 26) 220.
- [78] L. Lyu, X. Jin, Z. Li, S. Liu, Y. Li, R. Su, et al., TBBPA regulates calcium-mediated lysosomal exocytosis and thereby promotes invasion and migration in hepatocellular carcinoma, *Ecotoxicol. Environ. Saf.* 192 (2020 Apr) 110255.
- [79] C.B. Eding, J. Domert, P. Wäster, F. Jerhammar, I. Rosdahl, K. Öllinger, Melanoma growth and progression after ultraviolet A irradiation: impact of lysosomal exocytosis and cathepsin proteases, *Acta Derm. Venereol.* 95 (7) (2015 Sep) 792–797.
- [80] J. Ridinger, E. Koenke, F.R. Kolbinger, K. Koerholz, S. Mahboobi, L. Hellweg, et al., Dual role of HDAC10 in lysosomal exocytosis and DNA repair promotes neuroblastoma chemoresistance, *Sci. Rep.* 8 (2018 Jul 3) 10039.
- [81] M.S. Kim, S.H. Yang, M.S. Kim, TRPML3 enhances drug resistance in non-small cell lung cancer cells by promoting Ca²⁺-mediated lysosomal trafficking, *Biochem. Biophys. Res. Commun.* 627 (2022 Oct 30) 152–159.
- [82] E.R. Machado, D. van de Vlekkert, H.S. Sheppard, S. Perry, S.M. Downing, J. Laxton, et al., Haploinsufficiency of the lysosomal sialidase NEU1 results in a model of pleomorphic rhabdomyosarcoma in mice, *Commun. Biol.* 5 (1) (2022 Sep 20) 992.
- [83] M.S. Kim, S.H. Yang, M.S. Kim, Role of ABCA2 and its single nucleotide polymorphisms (4873T>A and 4879G>C) in the regulation of multi-drug resistance in oral squamous carcinoma cells, *Biochem. Biophys. Res. Commun.* 666 (2023 Jul 23) 1–9.
- [84] Y. Liu, Y. Zhou, K. Zhu, Inhibition of glioma cell lysosome exocytosis inhibits glioma invasion, in: G. Velasco (Ed.), *PLoS One* 7 (9) (2012 Sep 28) e45910.
- [85] B. Kim, G. Kim, H. Kim, Y.S. Song, J. Jung, Modulation of Cisplatin Sensitivity through TRPML1-mediated lysosomal exocytosis in Ovarian cancer cells: a comprehensive metabolomic approach, *Cells* 13 (2) (2024 Jan 8) 115.
- [86] R. Chen, M. Jäättelä, B. Liu, Lysosome as a central Hub for rewiring PH homeostasis in tumors, *Cancers* 12 (9) (2020 Aug 27) 2437.
- [87] N.S. Soysa, N. Alles, Osteoclast function and bone-resorbing activity: an overview, *Biochem. Biophys. Res. Commun.* 476 (3) (2016 Jul 29) 115–120.
- [88] R.K. Singh, A.S. Haka, A. Asmal, V.C. Barbosa-Lorenzi, I. Grosheva, H.F. Chin, et al., TLR4 (Toll-Like receptor 4)-dependent signaling drives extracellular catabolism of LDL (Low-Density lipoprotein) aggregates, *Arterioscler. Thromb. Vasc. Biol.* 40 (1) (2020 Jan) 86–102.
- [89] M.I. Yuseff, A. Reversat, D. Lankar, J. Diaz, I. Fanget, P. Pierobon, et al., Polarized secretion of lysosomes at the B cell synapse couples antigen extraction to processing and presentation, *Immunity* 35 (3) (2011 Sep 23) 361–374.
- [90] L. Xu, D. Fukumura, R.K. Jain, Acidic extracellular pH induces vascular endothelial growth factor (VEGF) in human glioblastoma cells via ERK1/2 MAPK

- signaling pathway: mechanism of low pH-induced VEGF, *J. Biol. Chem.* 277 (13) (2002 Mar 29) 11368–11374.
- [91] P. Knopf, D. Stowbur, S.H.L. Hoffmann, N. Hermann, A. Maurer, V. Bucher, et al., Acidosis-mediated increase in IFN- γ -induced PD-L1 expression on cancer cells as an immune escape mechanism in solid tumors, *Mol. Cancer* 22 (1) (2023 Dec 15) 207.
- [92] M.M. Audero, T.M.A. Carvalho, F.A. Ruffinatti, T. Loeck, M. Yassine, G. Chinigò, et al., Acidic growth conditions promote epithelial-to-mesenchymal transition to select more aggressive PDAC cell phenotypes in vitro, *Cancers* 15 (9) (2023 Apr 30) 2572.
- [93] M. Damaghi, R. Gillies, Phenotypic changes of acid-adapted cancer cells push them toward aggressiveness in their evolution in the tumor microenvironment, *Cell Cycle Georget Tex* 16 (19) (2017 Oct 2) 1739–1743.
- [94] F. Erra Díaz, E. Dantas, J. Geffner, Unravelling the interplay between extracellular acidosis and immune cells, *Mediators Inflamm* 2018 (2018) 1218297.
- [95] D.A. Martinez, A.C. Vailas, R. Vanderby, R.E. Grindeland, Temporal extracellular matrix adaptations in ligament during wound healing and hindlimb unloading, *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 293 (4) (2007 Oct) R1552–R1560.
- [96] J. Tong, W.N. Wu, X. Kong, P.F. Wu, L. Tian, W. Du, et al., Acid-sensing ion channels contribute to the effect of acidosis on the function of dendritic cells, *J Immunol Baltim Md* 186 (6) (1950. 2011 Mar 15) 3686–3692.
- [97] M. Vermeulen, M. Giordano, A.S. Trevani, C. Sedlik, R. Gamberale, P. Fernández-Calotti, et al., Acidosis improves uptake of antigens and MHC class I-restricted presentation by dendritic cells, *J Immunol Baltim Md* 172 (5) (1950. 2004 Mar 1) 3196–3204.
- [98] A. El-Kenawi, C. Gatenbee, M. Robertson-Tessi, R. Bravo, J. Dhillon, Y. Balagurunathan, et al., Acidity promotes tumour progression by altering macrophage phenotype in prostate cancer, *Br. J. Cancer* 121 (7) (2019 Oct 1) 556–566.
- [99] A. Calcinotto, P. Filipazzi, M. Grioni, M. Iero, A. De Milito, A. Ricupito, et al., Modulation of microenvironment acidity reverses anergy in human and murine tumor-infiltrating T lymphocytes, *Cancer Res.* 72 (11) (2012 Jun 1) 2746–2756.
- [100] B. Fischer, B. Müller, K.G. Fischer, N. Baur, W. Kreutz, Acidic pH inhibits non-MHC-restricted killer cell functions, *Clin Immunol Orlando Fla* 96 (3) (2000 Sep) 252–263.
- [101] T. Yadati, T. Houben, A. Bitorina, R. Shiri-Sverdlov, The ins and outs of cathepsins: physiological function and role in disease Management, *Cells* 9 (7) (2020 Jul 13) 1679.
- [102] B. Zhitomirsky, Y.G. Assaraf, Lysosomal accumulation of anticancer drugs triggers lysosomal exocytosis, *Oncotarget* 8 (28) (2017 Jul 11) 45117–45132.
- [103] E. Liaudet-Coopman, M. Beaujouin, D. Derocq, M. Garcia, M. Glondu-Lassis, V. Laurent-Matha, et al., Cathepsin D: newly discovered functions of a long-standing aspartic protease in cancer and apoptosis, *Cancer Lett.* 237 (2) (2006 Jun 18) 167–179.
- [104] M.M. Mohamed, B.F. Sloane, Cysteine cathepsins: multifunctional enzymes in cancer, *Nat. Rev. Cancer* 6 (10) (2006 Oct) 764–775.
- [105] D.R. Sudhan, D.W. Siemann, Cathepsin L inhibition by the small molecule KGP94 suppresses tumor microenvironment enhanced metastasis associated cell functions of prostate and breast cancer cells, *Clin. Exp. Metastasis* 30 (7) (2013 Oct) 891–902.
- [106] S.T. Kundu, C.L. Grzeskowiak, J.J. Fradette, L.A. Gibson, L.B. Rodriguez, C. J. Creighton, et al., TMEM106B drives lung cancer metastasis by inducing TFEB-dependent lysosome synthesis and secretion of cathepsins, *Nat. Commun.* 9 (1) (2018 Jul 16) 2731.
- [107] H.R. Seo, S. Bae, Y.S. Lee, Radiation-induced cathepsin S is involved in radioresistance, *Int. J. Cancer* 124 (8) (2009 Apr 15) 1794–1801.
- [108] X. Zhang, J. Wang, X. Li, D. Wang, Lysosomes contribute to radioresistance in cancer, *Cancer Lett.* 439 (2018 Dec 28) 39–46.
- [109] I.V. Karagounis, D. Kalamida, A. Mitrakas, S. Pouliliou, M.V. Liouisa, A. Giatromanolaki, et al., Repression of the autophagic response sensitises lung cancer cells to radiation and chemotherapy, *Br. J. Cancer* 115 (3) (2016 Jul) 312–321.
- [110] P.H. Wu, Y. Onodera, A.J. Giaccia, Q.T. Le, S. Shimizu, H. Shirato, et al., Lysosomal trafficking mediated by Arl8b and BORC promotes invasion of cancer cells that survive radiation, *Commun. Biol.* 3 (1) (2020 Oct 27) 620.
- [111] K.J. Gotink, H.J. Broxterman, M. Labots, R.R. de Haas, H. Dekker, R.J. Honeywell, et al., Lysosomal sequestration of sunitinib: a novel mechanism of drug resistance, *Clin Cancer Res Off J Am Assoc Cancer Res.* 17 (23) (2011 Dec 1) 7337–7346.
- [112] M.S. Kim, S. Muallem, S.H. Kim, K.B. Kwon, M.S. Kim, Exosomal release through TRPML1-mediated lysosomal exocytosis is required for adipogenesis, *Biochem. Biophys. Res. Commun.* 510 (3) (2019 Mar 12) 409–415.
- [113] L. Ermini, A. Farrell, S. Alahari, J. Ausman, C. Park, J. Sallais, et al., Ceramide-induced lysosomal biogenesis and exocytosis in early-onset Preeclampsia promotes exosomal release of SMPD1 Causing endothelial Dysfunction, *Front. Cell Dev. Biol.* 9 (2021 May 4) 652651.
- [114] C. Le Guilcher, I. Garcin, O. Dellis, F. Cauchois, A. Tebbi, I. Doignon, et al., The P2X4 purinergic receptor regulates hepatic myofibroblast activation during liver fibrogenesis, *J. Hepatol.* 69 (3) (2018 Sep) 644–653.
- [115] J. Xu, R. Camfield, S.M. Gorski, The interplay between exosomes and autophagy - partners in crime, *J. Cell Sci.* 131 (15) (2018 Aug 3) jcs215210.
- [116] W.H. Chang, R.A. Cerione, M.A. Antonyak, Extracellular vesicles and their roles in cancer progression, *Methods Mol Biol Clifton NJ* 2174 (2021) 143–170.
- [117] X. Zhou, Y. Jia, C. Mao, S. Liu, Small extracellular vesicles: non-negligible vesicles in tumor progression, diagnosis, and therapy, *Cancer Lett.* 580 (2024 Jan) 216481.
- [118] Y. Pan, Q. Zhao, H. He, Y. Qi, Y. Bai, J. Zhao, et al., TRPML1 as a potential therapeutic target for triple-negative breast cancer: a review, *Front. Oncol.* 13 (2023) 1326023.
- [119] W. Du, M. Gu, M. Hu, P. Pinchi, W. Chen, M. Ryan, et al., Lysosomal Zn²⁺ release triggers rapid, mitochondria-mediated, non-apoptotic cell death in metastatic melanoma, *Cell Rep.* 37 (3) (2021 Oct 19) 109848.
- [120] J.M. Lankelma, D.M. Voorend, T. Barwari, J. Koetsveld, A.H. Van der Spek, A.P.N. A. De Porto, et al., Cathepsin L, target in cancer treatment? *Life Sci.* 86 (7–8) (2010 Feb 13) 225–233.
- [121] A. Domagala, K. Fidy, M. Bobrowicz, J. Stachura, K. Szczygiel, M. Firczuk, Typical and Atypical Inducers of lysosomal cell death: a promising anticancer Strategy, *Int. J. Mol. Sci.* 19 (8) (2018 Aug 1) 2256.