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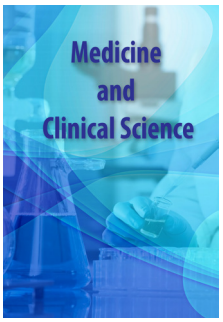

Exploring the Functional Significance of Lysosomes in Cancer Drug Resistance

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Abstract

Chemotherapy stands as a primary therapeutic approach for tackling a spectrum of malignancies. Nonetheless, the emergence of resistance to chemotherapeutic agents poses a significant hurdle in achieving curative cancer treatments. Lysosomes, recognized as acidic cellular organelles predominantly engaged in intracellular digestion, have garnered increasing attention due to their implications in cancer biology. Notably, their relevance to cancer manifests in several ways: Firstly, the extracellular release of lysosomal enzymes actively promotes tumorigenesis. Secondly, the leakage of lysosomal hydrolases into the cytosol induces apoptosis. Lastly, weak chemotherapeutic bases, upon traversing the lysosomal membrane, become sequestered within lysosomes while adopting a cationic state. This sequestration of drugs within lysosomes diminishes their cytotoxic potential, restricts their availability at target sites, and contributes significantly to the development of drug resistance in cancer. This review comprehensively explores diverse mechanisms underpinning lysosomal drug sequestration and delves into their repercussions on the phenomenon of multidrug resistance in cancer. Furthermore, we delve into strategies aimed at surmounting drug resistance by leveraging lysosomes as subcellular targets, with the aim of reversing drug sequestration and thwarting drug resistance in the context of cancer therapy.

Introduction

Lysosomes constitute membranous compartments inherent to eukaryotic cells, present across mammalian cell types, except in mature erythrocytes, and were initially unveiled in 1955 by Christian de Duve [1]. These minute vesicular structures, exhibiting a diameter of 0.25-1 μm, are encased by a specialized lipid-protein bilayer, measuring 7-10 nm in thickness [2]. Lysosomes accommodate in excess of 50 distinct hydrolytic enzymes, encompassing acid hydrolases such as proteases, nucleases, glycosidases, lipases, phospholipases, phosphatases, peptidases, and sulfatases [3]. Originating in the rough endoplasmic reticulum, these enzymes undergo synthesis, subsequent modification, and subsequent translocation to primary lysosomes [4]. The optimal pH range of 4.5-5.5, conducive to lysosomal enzymatic activities, is primarily upheld by the V-type ATPase (H+-ATPase) complex. This intricate assembly operates through ATP-driven proton transport from the cytosol to the lysosomal lumen [5]. The lysosomal membrane effectively segregates these enzymes from the cytoplasm; in the event of membrane disruption and the consequent release of lysosomal content into the cytoplasm (characterized by a pH of 7.2-7.3), the enzymatic hydrolytic capacity is diminished, with minimal detrimental effects on other cytoplasmic components [6]. Foremost among their functions, lysosomes partake in the degradation of proteins, nucleic acids, lipids, and carbohydrates through the orchestrated involvement of lysosomal hydrolases. These organelles are pivotal in the digestion and recycling of cellular macromolecules, dysfunctional organelles, and a portion of the cytoplasm via the process of autophagy. Additionally, lysosomes engage in the degradation of extracellular material acquired through endocytosis and phagocytosis. Consequently, lysosomes play a pivotal role in maintaining cellular homeostasis [7]. Modern perspectives attribute multifaceted roles to lysosomal enzymes, predominantly cathepsins, including their involvement in bone remodeling, prohormone processing, angiogenesis, cellular apoptosis, and the invasive behavior of cancer cells [8].

The involvement of lysosomes in the progression of cancer

Rapidly proliferating neoplastic cells rely significantly on the optimal functionality of lysosomes, and the progression of cancer cells is distinguished by substantial alterations within the lysosomal compartment in comparison to

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normal cells [9]. These alterations encompass the relocation of lysosomes from the perinuclear domain to the periphery (a phenomenon possibly linked to the presence of acidic pH in the extracellular milieu) and modifications in the quantity and size of lysosomes. Furthermore, an elevation in the expression, secretion, and/or activation of lysosomal enzymes, including cathepsins, is observed [9]. A majority of these changes exhibit close associations with invasive proliferation, angiogenesis, and resistance to therapeutic agents [10]. Lysosomes are implicated in a dual capacity in the process of cancer development [11,12], as they can exert influence over both invasive tumor expansion and vascular growth [13], alongside affording protection to malignant cells against certain chemotherapeutic compounds, thereby potentially contributing to the emergence of drug resistance [3].

Lysosomes, functioning as organelles that harbor intracellular calcium ions, also partake in various cellular processes, with disruptions in calcium homeostasis correlating to diverse diseases [14]. This is attributed to the participation of Ca2+-permeable mucolipin channels containing transient receptor potential (TRP) domains (TRPML, TRPML1-3), which integrate cellular growth, division, and metabolism. Consequently, dysregulation of TRPML activity holds significance in cancer progression [15,16]. In the tumor microenvironment, activated autophagy serves cancer cells in the degradation of superfluous or impaired proteins and cellular organelles, catering to augmented energy and nutrient needs [17]. TRPMLs are also implicated in the regulation of lysosomal function and autophagic processes by releasing intracellular calcium [18]. TRPML1 notably activates the calmodulin (CaM)/CaMKKβ/AMPK pathway (stimulating autophagosome formation) and CaM/CaN/TFEB pathway (facilitating protein delivery to lysosomes), maintains mTORC1 activity (averting tumor cell demise and promoting lysosomal modifications), enhances lysosomal degradation functions, and supports Syt7-dependent lysosomal exocytosis [19]. These contributions lead to modifications in the tumor microenvironment, degradation of extracellular matrix (ECM) constituents, and consequently, heightened tumor advancement [19].

Lysosomes as facilitators of multi drug resistance in cancer

The involvement of lysosomes in chemoresistance is linked to actions such as those of P-glycoprotein (P-gp), a member of the ATP-binding cassette (ABC) transporter B subfamily responsible for expelling various substances, including drugs, from cells [20]. Recent findings suggest that overexpression of P-gp within lysosomes is witnessed in resistant cancer cells, a result of incorporation into lysosomal membranes during recycling rather than redistribution post de novo synthesis [21,22]. Cancer cells expressing MDR multidrug transporters efficiently eliminate lysosomotropic ionizing drugs, sequestering them in lysosomes and then releasing them via exocytosis [22]. Accumulation of these drugs within lysosomes primarily results from ion trapping or active transport [23]. Given the comparatively weaker lysosomal membranes of cancer cells versus normal cells, there is potential to selectively sensitize cancer cells to diverse forms of cell death, including apoptosis and autophagy, both of which hold therapeutic significance [20].

Sequestering anticancer agents originating from the hydrophobic weak base of lysosomes

Anticancer drugs derived from hydrophobic weak bases (such as sunitinib, doxorubicin, daunorubicin, mitoxantrone, imidazoacridinone, etc.) exhibit facile translocation across both hydrophobic cell membranes and lysosomal membranes. Nevertheless, upon entry into the lysosome, these anticancer agents undergo a conversion to a charged state, induced by acidic proton ions, impairing their translocation to the cytoplasm and resulting in their accumulation within the lysosome. Consequently, their anticancer functionality is compromised [24]. Given the prevalence of more numerous and larger lysosomes in most cancer cells in comparison to normal cells, they capture a higher quantity of anticancer drugs, even when exposed to equivalent drug concentrations, thus endowing resistance to anticancer agents [25]. Consequently, lysosomal malfunction and membrane compromise, primarily through lysosomal membrane permeabilization (LMP), can precipitate the efflux of sequestered anticancer agents, enabling their interaction with other organelles. Ultimately, this process enhances sensitivity and culminates in the demise of cancer cells.

The sequestration of anticancer drugs mediated by ATP-binding cassette (ABC) transporters is a pivotal phenomenon

ABC transporters, prominently localized in the plasma membrane, exhibit the remarkable ability to identify and extrude anticancer agents striving to infiltrate neoplastic cells, thereby instigating resistance to a spectrum of anticancer pharmacotherapies [26]. These ABC transporters are further distributed within the lysosomal membrane, thus facilitating the translocation of anticancer agents from the cytoplasm into lysosomes, subsequently culminating in the accumulation of these therapeutic compounds [27]. Notably, P-gp recognized for its capability to discern chemotherapeutic agents and induce multidrug resistance in malignant neoplasms, exhibits a distinctive subcellular distribution. It is not only confined to the plasma membrane but also found within the membranes of diverse intracellular organelles. This versatile localization results in the intracellular sequestration of anticancer drugs such as daunorubicin and doxorubicin within lysosomes and other organelles, thereby conferring specific protection against these anticancer agents [22]. Another member of the transporter family, ABCA3, is primarily situated within lysosomes and plays a pivotal role in mediating anticancer drug resistance through the entrapment of drugs like daunorubicin and imatinib within the lysosomal milieu. The absence of ABCA3 expression augments sensitivity to anticancer drugs [28].

Approaches to reverse lysosomal sequestration

Investigation of alkalinizing agents for the reversal of lysosomal drug sequestration

The present study postulates multiple mechanisms to potentially counteract the phenomenon of lysosomal drug sequestration. In the ensuing sections, we explore prospective strategies aimed at facilitating the translocation of drugs from the lysosomal lumen to the cytosol. Such translocation is envisaged to enhance drug accessibility to their intended target sites. One effective approach to reversing the lysosomal accumulation of drugs, particularly weak chemotherapeutic bases, is through the use of lysosome alkalinizing agents [29]. Bafilomycin A1, an inhibitor of vesicular H+-ATPase, has been demonstrated to alkalize lysosomes and mitigate drug sequestration [30]; however, its in vivo applicability is hindered by its pronounced toxicity. A more viable alkalinizing agent is chloroquine,

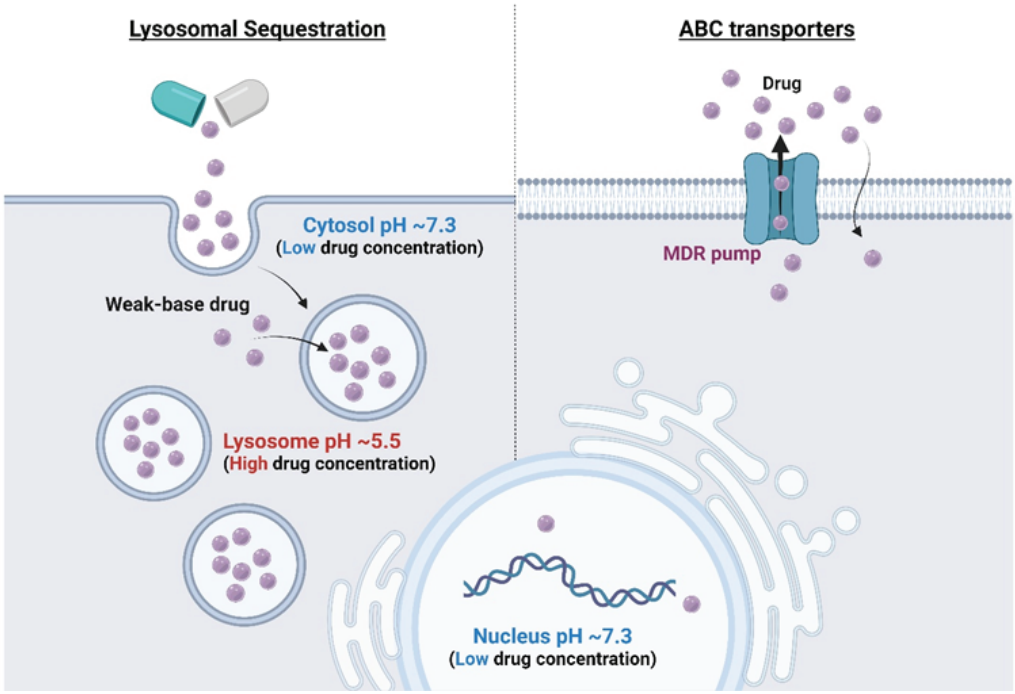

Figure 1. *Lysosomal drug sequestration. Hydrophobic weak-base compounds exhibit unhindered diffusion across both the plasma membrane and the lysosomal membrane. However, upon interaction with the acidic environment within the lysosomal lumen, these pharmaceutical agents undergo protonation, rendering them incapable of traversing the lipid bilayer of the lysosomal membrane. Consequently, this phenomenon leads to the sequestration of these drugs within the lysosomes, ultimately culminating in a diminished concentration of the drug within the cytoplasm and the cell nucleus. Members of the ABC superfamily of transport proteins are situated within the plasma membrane, where they play a pivotal role in expediting the efflux of drugs from the cellular environment. Furthermore, certain ABC transporters have been identified within lysosomal membranes, actively mediating the transport of drugs into lysosomes, thereby promoting the sequestration of drugs within these organelles.*

which has shown promise in inhibiting lysosomal function by elevating lysosomal pH [31]. Studies involving chloroquine administration in mice support its potential to disrupt lysosomal drug sequestration. Moreover, chloroquine has exhibited synergistic effects with chemotherapeutic agents, exemplified by enhanced cytotoxicity when combined with doxorubicin in liver carcinoma cells. Additionally, the prevention of subcellular drug trapping by lysosomal alkalinization has been observed to abolish drug resistance [32]. These approaches are plausible due to discernible pH gradient disparities between multidrug-resistant cancer cells and their wild-type, drug-sensitive counterparts, suggesting that the judicious use of well-tolerated alkalinizing agents may circumvent lysosomal drug sequestration, consequently augmenting the efficacy of cytotoxic drugs [33].

Utilizing lysosomotropic agents to alleviate lysosomal drug sequestration

An alternative strategy to counteract lysosomal drug sequestration involves employing lipophilic drugs that, while prone to lysosomal sequestration, possess the capability to induce lysosomal membrane permeabilization (LMP) [34]. Chloroquine, a well-known lysosomotropic agent, has been reported to enhance cytotoxicity and synergize with chemotherapeutic drugs. Chloroquine, by triggering lysosomal membrane destabilization in various tumor cells, has demonstrated the potential to restore sensitivity to cisplatin in refractory non-small-cell lung cancer cells [35] and potentiate the cytotoxic effects of topotecan by inhibiting autophagy [36]. Another compound, di-2-pyridylketone 4,4-dimethyl-3-thiosemicarbazone (Dp44mT), has been found to accumulate within tumor cell lysosomes, where it induces LMP [37]. Within the lysosome, Dp44mT forms a copper complex capable of generating cytotoxic reactive oxygen species (ROS), thereby triggering LMP [37].

Harnessing conjugates to overcome lysosomal drug sequestration

To mitigate lysosomal drug sequestration, another approach involves the conjugation of chemotherapeutic drugs with acid-labile compounds. Hydrazone, a frequently employed linker molecule for this purpose, exhibits stability at cytosolic pH and undergoes hydrolysis at lysosomal pH [38]. Supporting evidence comes from a study in which doxorubicin was conjugated to polyamidoamine dendrimers via hydrazone [39], resulting in the release of doxorubicin into the nucleus and the induction of cell death.

Lysosomal photodestruction as a strategy for reversing lysosomal sequestration

An innovative approach to reversing lysosomal drug sequestration entails lysosomal photodestruction of weakly basic chemotherapeutics that also exhibit fluorescence properties. This approach has been shown to lead to cell lysis through the generation of reactive oxygen species (ROS) following the photodestruction of imidazoacridinone-loaded

lysosomes in multidrug-resistant cancer cells [40]. In certain studies, combination of sunitinib and phototherapy has been employed to combat lysosomal drug localization [41]. However, it is worth noting that this approach is limited in its utility due to its superficial and localized nature.

Conclusion

In spite of extensive endeavors aimed at enhancing the efficacy of chemotherapy, the persistence of treatment failure and the emergence of resistance mechanisms continue to pose substantial challenges. For instance, in the case of the P-glycoprotein (P-gp) efflux pump, which can significantly curtail the effectiveness of cytostatic drugs, there are presently no clinically approved therapeutic interventions available. As comprehensively outlined within this review, the involvement of lysosomes in drug resistance has inaugurated a novel research domain within the realm of multidrug resistance, with the objective of surmounting chemoresistance. This involvement encompasses not only direct lysosomal processes but also extends to lysosome-associated signaling pathways. Substantial evidence substantiates the proposition that modulating lysosomal function may represent a promising strategy for enhancing chemotherapy sensitization, thereby influencing a multitude of survival-promoting mechanisms. These mechanisms include the modulation of efflux transporter trafficking, drug sequestration, and pathways regulated by transcription factor EB (TFEB), which encompass autophagy and DNA repair. Furthermore, the inhibition of lysosomal function holds the potential to counteract P-glycoprotein-mediated chemoresistance, instilling optimism for the prospective development of lysosome-targeted adjuvant therapies.

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