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Exploiting Lysosomal Vulnerabilities: A Novel Therapeutic Strategy to Induce Immunogenic Cell Death in Oncogene-Addicted Cancers

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Abstract

Background: A significant challenge in oncology is the development of therapies that selectively eliminate cancer cells while engaging the immune system for durable protection. Oncogene-driven transformation has been shown to rewire cellular metabolism and organelle function, creating unique dependencies. Lysosomes, traditionally considered mere degradative centers, are emerging as critical regulators of cell survival and death. We hypothesized that oncogenic transformation, particularly by drivers like Her2, creates a lysosomal "Achilles' heel," priming these organelles for permeabilization and offering a novel therapeutic target. **Methods:** Using the MCF-10A-neoT model of oncogene-driven transformation, we first characterized lysosomal alterations. We then conducted a high-throughput screen for compounds inducing Lysosomal Membrane Permeabilization (LMP), focusing on Reactive Oxygen Species (ROS) inducers. Lead compounds were evaluated for their ability to trigger established markers of Immunogenic Cell Death (ICD), including surface calreticulin (CRT), ATP release, and HMGB1 release. Selectivity and efficacy were further validated in co-culture models and immunocompetent mouse models. Results: Oncogene-transformed cells exhibited significantly enlarged lysosomes, altered subcellular distribution, and increased cathepsin B/L activity compared to their non-transformed counterparts. Our screen identified several ROS-generating compounds that selectively induced LMP in cancer cells. Treatment with these lead compounds resulted in the robust exposure of CRT, and the extracellular release of ATP and HMGB1, confirming the induction of ICD. In co-culture, the compounds demonstrated a clear therapeutic window, preferentially killing cancer cells. In vivo studies in immunocompetent mice confirmed tumor regression and the establishment of a protective anti-tumor immune memory. **Conclusion:** Our findings validate that targeted LMP induction is a viable strategy for selectively killing oncogene-addicted cancer cells. Moreover, by triggering ICD, this approach not only directly eliminates tumor cells but also stimulates a systemic immune response, addressing the critical need for therapies that provide long-term anti-cancer immunity.

Introduction

Cancer remains a leading cause of mortality worldwide, with resistance to conventional therapies and tumor recurrence representing major clinical hurdles [1]. The paradigm of cancer treatment has progressively shifted from broadly cytotoxic agents to targeted therapies that exploit specific molecular vulnerabilities within cancer cells [2]. The concept of "oncogene addiction," where cancer cells become dependent on a single activated oncogenic pathway for survival and proliferation, has been a cornerstone of this approach [3]. While therapies targeting these pathways, such as tyrosine kinase inhibitors, have shown remarkable success, their efficacy is often limited by the development of resistance [4].

Concurrently, the success of immunotherapy has underscored the critical role of the host immune system in achieving durable cancer control [5]. However, many tumors are "cold"or immunologically silent, failing to be recognized and attacked by T cells [6]. A promising strategy to overcome this is the use of therapeutics that not only kill cancer cells but also do so in a way that stimulates an immune response—a process known as immunogenic cell death (ICD) [7]. ICD is characterized by the spatiotemporal emission of damage-associated molecular patterns (DAMPs), including the pre-apoptotic exposure of calreticulin (CRT) on the cell surface, and the release of ATP and high-mobility group box 1 (HMGB1) protein [8]. These signals act as "eat me" flags and chemoattractants for antigen-presenting cells, ultimately leading to the priming of tumor-specific T cells [9].

Lysosomes, acidic organelles containing a battery of hydrolytic enzymes, are essential for cellular degradation and recycling.

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Beyond their catabolic role, they are integral to cell death pathways [10]. Lysosomal membrane permeabilization (LMP) results in the leakage of cathepsins and other hydrolases into the cytosol, initiating caspase-dependent and -independent cell death [11]. Notably, cancer cells often exhibit altered lysosomal biology, including increased lysosomal biogenesis, enlarged size, and elevated cathepsin levels, which are thought to support their high metabolic demands and invasive potential [12]. Paradoxically, these very adaptations may render cancer lysosomes more fragile and susceptible to LMP, representing a therapeutically exploitable vulnerability [13].

In this study, we propose that oncogene addiction extends to lysosomal homeostasis. We posit that oncogenic drivers, such as Her2, rewire lysosomal function, creating a primed state that is uniquely susceptible to permeabilization. We further hypothesize that targeted induction of LMP in these oncogene-addicted cells will not only trigger direct cell death but will do so in an immunogenic manner, leading to the selective elimination of tumors and the stimulation of a protective anti-tumor immune response.

Materials and Methods

❖ Cell Culture and Models

Non-transformed human mammary epithelial cells (MCF-10A) and their oncogene-transformed counterparts (MCF-10A-neoT, expressing activated Her2/neu) were cultured as previously described [14]. All cell lines were regularly tested for mycoplasma contamination.

❖ Lysosomal Characterization

Lysosomes were visualized by immunofluorescence staining for LAMP-1. Lysosomal size and intracellular distribution were quantified using ImageJ software from at least 100 cells per group. Cathepsin B and L activities were measured using fluorogenic substrates (Sigma-Aldrich) according to the manufacturer's protocols.

❖ Compound Screening for LMP Induction

A library of 2,000 bioactive compounds, with an emphasis on known ROS inducers, was screened. Cells were treated with compounds (10 µM) for 24 hours. LMP was assessed using the acridine orange (AO) relocation assay, where a loss of red lysosomal fluorescence indicates lysosomal leakage [15]. Viability was assessed in parallel using the MTT assay.

❖ Assessment of Immunogenic Cell Death Markers

Surface Calreticulin: Treated cells were stained with an anti-CRT antibody and analyzed by flow cytometry.

ATP Release: Extracellular ATP in cell culture supernatants was quantified using an ENLITEN® ATP Assay Kit (Promega).

HMGB1 Release: HMGB1 levels in supernatants were measured using a commercial ELISA kit (Tecan).

❖ Selectivity and Co-culture Assays

Cancer cells (MCF-10A-neoT) were co-cultured with non-transformed MCF-10A cells at a 1:1 ratio. Cells were treated with lead compounds, and selective killing was assessed by flow cytometry using cell-specific fluorescent markers (CellTracker™ dyes).

❖ In Vivo Studies

All animal procedures were approved by the Institutional Animal Care and Use Committee. Immunocompetent BALB/c mice were inoculated with syngeneic CT26.WT tumors. Once tumors reached ~100 mm^3, mice were randomized into treatment groups (n=8) and administered either vehicle or lead compound (10 mg/kg, i.p., every other day). Tumor volume and mouse weight were monitored. For immune memory studies, surviving mice were re-challenged with the same tumor cells on the opposite flank.

Results

❖ Oncogenic Transformation Alters Lysosomal Phenotype

Consistent with our hypothesis, MCF-10A-neoT cells displayed a significantly altered lysosomal compartment compared to parental MCF-10A cells. Lysosomes in transformed cells were notably enlarged and exhibited a perinuclear clustering pattern (Figure 1A). Quantification revealed a 2.5-fold increase in mean lysosomal volume ($p < 0.001$) (Figure 1B). Furthermore, cathepsin B/L activity was elevated by over 3-fold in the oncogene-addicted cells ($p < 0.001$) (Figure 1C), confirming a state of lysosomal hyperactivity.

❖ Identification of LMP-Inducing Compounds with Selective Toxicity

Our compound screen identified 15 initial hits that induced LMP in MCF-10A-neoT cells without significant toxicity to MCF-10A cells at 10 µM. Three lead compounds, all of which are known to generate intracellular ROS, were selected for further study. Compound LMP-04, a quinone-based agent, showed the most pronounced selective effect, inducing LMP in over 70% of cancer cells versus less than 15% of normal cells (Figure 2A-B).

❖ LMP Induction Triggers Robust Immunogenic Cell Death

Treatment with lead compound LMP-04 resulted in a time-dependent increase in all key ICD markers. We observed a 5-fold increase in surface-exposed CRT within 6 hours of treatment (Figure 3A). Concurrently, there was a significant release of ATP (8-fold increase) and HMGB1 (6-fold increase) into the supernatant 24 hours post-treatment (Figure 3B-C).

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This coordinated emission of DAMPs confirms the immunogenic nature of the cell death triggered by LMP.

- **Lead Compound Demonstrates Selectivity and Engages the Immune System In Vivo**

In co-culture models, LMP-04 selectively eliminated MCF-10A-neoT cells with a selectivity index (SI) of >15, sparing the non-transformed counterparts (Figure 4A). In immunocompetent mouse models, treatment with LMP-04 led to significant tumor growth inhibition ($p < 0.01$) and complete regression in 50% of the mice (Figure 4B). Critically, upon tumor re-challenge, 100% of the cured mice rejected the new inoculum, while all naïve control mice developed tumors, demonstrating the establishment of protective immunological memory (Figure 4C).

Discussion

This study provides compelling evidence that the lysosomal compartment of oncogene-addicted cancer cells represents a druggable vulnerability that can be harnessed to induce both selective tumor cell killing and a potent anti-tumor immune response. Our initial findings that Her2-driven transformation leads to enlarged, cathepsin-rich lysosomes align with the concept that oncogenic signaling reprograms organellar biology to support rapid growth [12, 16]. This altered state, however, creates a latent fragility.

The success of our screen in identifying ROS-generating compounds as selective LMP inducers is mechanistically coherent. Cancer cells often exist in a state of elevated basal ROS, making them more vulnerable to further oxidative stress [17]. The lysosomal membrane is particularly rich in polyunsaturated fatty acids, making it a sensitive target for peroxidation, which can directly lead to permeabilization [18]. Our lead compound, LMP-04, effectively exploited this vulnerability, triggering LMP and the subsequent cascade of ICD.

The demonstration of a full ICD profile—CRT exposure, ATP release, and HMGB1 release—is a crucial finding. It bridges the gap between direct lysosome-mediated cytotoxicity and systemic immunity. By emitting these potent "danger signals," the dying cancer cells orchestrate their own phagocytosis and antigen presentation, effectively turning the tumor into an in-situ vaccine [9]. The in vivo results strongly support this, showing not only tumor regression but also the development of immune memory, a hallmark of successful ICD.

A key strength of this approach is its potential to overcome resistance mechanisms associated with direct oncogene inhibition. By targeting a downstream organellar consequence of oncogenic signaling rather than the oncoprotein itself, LMP induction may be effective against tumors that have developed resistance to targeted agents.

Conclusion

We have established a novel therapeutic paradigm centered on lysosome-targeted therapy. We demonstrate that targeted LMP induction is a viable and powerful strategy for selectively eliminating oncogene-addicted cancer cells via a highly immunogenic form of cell death. This dual mechanism of action—direct cytotoxicity and immune activation—addresses two critical challenges in oncology: selectivity and long-term protection. Future work will focus on optimizing lead compounds for clinical translation and identifying biomarkers to predict which patients and oncogene-addictions are most susceptible to this lysosome-targeted approach.

Conflict of Interest: NIL

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Declarations:

Author's Contribution:

- Conceptualization, data collection, interpretation, drafting of the manuscript and intellectual revisions
- The author agrees to take responsibility for every facet of the work, making sure that any concerns about its integrity or veracity are thoroughly examined and addressed

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