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Targeting the Mitochondrial Unfolded Protein Response in Cancer: Opportunities and Challenges

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Abstract

Increasing evidence indicates that a mitochondria-specific stress response referred to as the mitochondrial unfolded protein response (UPR^{mt}) is activated to maintain mitochondrial integrity and support tumor growth. In this forum article, we discuss the recent advances and current challenges in therapeutically targeting UPR^{mt} in cancer.

Keywords

Mitochondrial unfolded protein response; mitochondrial chaperonins; mitochondrial proteases; mitochondrial proteostasis; cancer

Mitochondrial Stress and Mitochondrial Unfolded Protein Response

Mitochondria are essential for tumor growth and progression. Mitochondria supply bioenergetic and biosynthetic demands during tumor growth through production of ATP by oxidative phosphorylation (OXPHOS); and synthesis of nucleotides, amino acids, and lipids [1]. Mitochondria are crucial for metabolic reprogramming to coordinate energy production during stressful conditions. However, an increased demand for mitochondrial activity by highly proliferating cells leads to excessive generation of reactive oxygen species (ROS), toxic byproducts of mitochondria. ROS induce oxidative damage and promote the unfolding/aggregation of proteins in mitochondria [2].

UPR^{mt}, best studied in *C. elegans*, is activated during mitochondrial stress to maintain mitochondrial health [3]. UPR^{mt} induces the expression of (1) chaperones that promote folding of mitochondrial proteins that have unfolded/aggregated due to excessive ROS, and (2) proteases that eliminate mitochondrial proteins that have been irreparably damaged by ROS (Figure 1). Lead chaperones include heat shock protein 60 (HSP60), heat shock protein

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10 (HSP10), and mitochondrial heat shock protein 70 (mtHSP70). Key proteases include Lon peptidase 1 (LONP1) and Caseinolytic protease (ClpP).

Studies of UPR^{mt} in cancer have mostly focused on the ability of individual UPR^{mt} components to promote tumor growth. Emerging evidence highlights the ability of UPR^{mt} as a whole to attenuate mitochondrial stress and support tumor growth *in vivo* [4, 5]. Given the tumor-supportive role of UPR^{mt}, various groups have endeavored to target UPR^{mt} proteins (Table 1). While many inhibitors of individual UPR^{mt} components are known, we highlight only those inhibitors that have either reached the clinical stage or show promising preclinical data.

Targeting UPR^{mt}-specific Chaperones in Cancer

HSP60/HSP10

HSP60 forms a complex with HSP10 to function as a key chaperone in mitochondria. Majority of HSP60/HSP10 inhibitors target HSP60 of this complex. Mizoribine, derived from *Eupenicillium brefeldianum*, was the first natural molecule identified as an HSP60 inhibitor [6]. Traditionally used as an immunosuppressant after renal transplantation, mizoribine was later shown to bind HSP60 and inhibit the chaperonin activity of HSP60/HSP10 in the mM range. Randomized, double-blind, placebo-controlled phase 1 trials enrolled healthy males for administration of mizoribine to determine the mizoribine tolerability. Values were compared by one-way analysis of variance (ANOVA) and mizoribine displayed a favorable safety profile, except for transient elevations in serum uric acid at the highest dose (12 mg/kg). However, mizoribine was unable to reach concentrations required to inhibit HSP60/HSP10 and peaked at plasma concentrations of ~30 μM in the clinic [6].

Epolactaene, isolated from *Penicillium* sp. BM 1689-P, and its synthetic derivatives selectively target HSP60 in the 2–4 μM range and induce cell cycle arrest in cancer cells [7]. Other synthetic molecules, such as gold (III) porphyrin complexes and BSP-SCA (5-sulfonamido-2-phenylbenzoxazole and salicylanilide) hybrid analogs, directly target HSP60 and exert anti-cancer effects [8, 9]. However, the specificity and efficacy of such compounds *in vivo* remain to be established. Future studies must explore these properties before such inhibitors can be tested in the clinic.

mtHSP70

Another crucial mitochondrial chaperone is mtHSP70. MKT 077, a rhodacyanine dye, accumulates in cancer mitochondria due to the increased electrochemical gradient of the mitochondrial membrane. MKT 077 disrupts interactions between mtHSP70 and Bag co-chaperones to reduce the chaperone activity of mtHSP70.

MKT 077 is cytotoxic in many preclinical models of human cancer. MKT 077 was subjected to a phase 1 clinical trial to treat patients with advanced solid tumors refractory to standard chemotherapies [10]. Tumors with an increased mitochondrial membrane gradient, such as colon, were included for treatment. The primary aim was to determine MTK 077 toxicity to patients. Comparisons were evaluated using a Student's *t*-test. Reversible renal toxicity

was frequently observed. Therefore, recruitment for this study was discontinued. However, this trial did demonstrate that MKT 077 can safely inhibit mitochondrial functions and that rhodacyanine analogues with a higher therapeutic index could plausibly target mtHSP70 in cancer.

Structure-based design identified new rhodacyanine analogues, such as JG-231, that display increased selectivity and bioavailability, and are well-tolerated *in vivo* [11]. However, further challenges lie ahead as these rhodacyanine analogues are light sensitive, and difficult to synthesize and solubilize.

Targeting UPR^{mt}-specific Proteases in Cancer

LONP1

Triterpenoids isolated from plants and synthetic derivatives, such as CDDO-Me, block LONP1-mediated proteolysis in cancer cells and induce apoptosis [12]. A non-randomized, phase 1 clinical study of CDDO-Me enrolled advanced solid tumors and lymphoma patients refractory to standard therapy (NCT00508807)[I]. The purpose was to determine the dose-limiting toxicities and maximum tolerated dose, in order to guide dosing for phase 2 studies. Dose escalation by accelerated titration within single-patient cohorts was performed until grade-2 adverse events were observed. Upon completion of this study, comparisons were evaluated using a Student's *t*-test. Common adverse events included fatigue and nausea. The maximum tolerated dose was 900 mg/d and patients who received 1300 mg/d displayed reversible hepatotoxicity.

Although CDDO-Me targets LONP1, it also inhibits PPAR-γ, ubiquitin-specific-processing protease 7, and IκB kinase to exert its anti-cancer functions [12]. CDDO-Me was selected for clinical trials not simply based on its ability to inhibit LONP1 of the UPR^{mt} response, but for its inhibition of a range of tumor-supportive pathways. This precludes the therapeutic use of triterpenoids as LONP1-specific inhibitors. Interestingly, CDDO-Me also increased glomerular filtration rate. Consequently, CDDO-Me is now enrolling patients for clinical trials to treat chronic kidney disease (NCT03749447, NCT04702997)[II, III].

ClpP

The imipridone molecule ONC201 binds and hyperactivates ClpP, leading to excessive proteolysis, mitochondrial failure, and cell death in multiple cancer cell lines. Patients with advanced solid tumors and resistant to standard therapies (NCT02250781)[IV] were enrolled in an open label, single group assignment, phase 1 dose-escalation study for ONC201. The primary objective was to determine the safety and tolerability of ONC201 helping establish doses for phase 2 studies. Comparisons were evaluated upon completion of the study using descriptive statistics. Adverse events included cases of nausea and emesis. Overall, results indicated a favorable safety profile at the recommended phase 2 dose of 625 mg per week.

However, in addition to hyperactivating ClpP, ONC201 is also known to inactivate AKT/ERK signaling and inhibit dopamine receptor D2. In fact, the ability of ONC201 to hyperactivate ClpP was not known until after the clinical trial had commenced. The non-selective nature of ONC201 prevents its sole designation as a ClpP-targeting agent.

Notably, inhibition of ClpP activity by (3RS,4RS)-3-(non-8-en-1-yl)-4-(2-(pyridin-3-yl)ethyl)oxetan-2-one (A2-32-01) also shows beneficial effects in a preclinical model of leukemia [13]. However, the specificity and bioavailability of A2-32-01 in humans remain unknown and must be addressed before A-32-01 can be used for clinical trials.

Targeting UPR^{mt} Transcription Factors

A family of leucine zipper proteins initiate the transcription of chaperones and proteases during the UPR^{mt} response. Activating transcription factor 5 (ATF5) and CCAAT-enhancer-binding proteins (CEBPs) are key transcription factors, which exert their function either via homodimerization or heterodimerization. ATF5 is crucial for upregulating the transcription of UPR^{mt} components during mitochondrial stress [3], maintains mitochondrial proteostasis, and allows mitochondria to promote tumor growth and progression.

ATF5 protein was modified so that the DNA binding domain was mutated to eliminate transcriptional activity [14]. However, with the leucine zipper left intact, this dominant-negative (d/n)-ATF5 could bind the DNA interaction domains of ATF5, CEBPB, and CEBPD, and prevent their dimerization. Later, d/n-ATF5 was fused to cell-penetrating domains to produce (d/n)-ATF5 peptides that can be administered via injection to selectively trigger apoptosis in a broad range of treatment-resistant tumors in mice [14]. Such potent and tumor-selective effects allow (d/n)-ATF5 peptides to be ideal candidates for clinical studies.

Final Remarks

Altough UPR^{mt} is an attractive therapeutic target, many outstanding questions including the mechanism of UPR^{mt} activation in cancer and how does UPR^{mt} interact with oncogenes/tumor suppressors require further investigation.

Many challenges remain as the current inhibitors of UPR^{mt} appear to have issues with potency, specificity, and toxicity. As a result, only a few of these inhibitors have entered clinical trials and none have yet been approved for cancer therapy. These issues could eventually be overcome by the use of *in silico* screening and X-ray crystallography to guide the development of agents, which selectively target UPR^{mt} proteins. Drug uptake can be enhanced by the combined use of lipid nanoparticles for tumor cell delivery and attachment of cationic groups, which utilize high transmembrane potential of the mitochondrial membrane for mitochondrial targeting [15].

The success of inhibiting UPR^{mt} as a form of therapy likely correlates with the dependency of individual tumors upon UPR^{mt} for growth and survival. Genomic profiling of tumors for signs of mitochondrial stress and dysfunction could help predict the efficacy of targeting UPR^{mt}. In addition, tumor cells that emerge after chemotherapy are highly dependent upon mitochondria for proliferation. Optimal timing and combinational use of UPR^{mt} inhibitors with standard therapies could enhance current care [15]. Overall, cancer dependency on UPR^{mt} provides the vast, and untapped potential of appropriately targeting this response for the treatment and management of cancer burden.

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Resources

I. This study is registered with ClinicalTrials.gov under the following link: https://clinicaltrials.gov/ct2/show/NCT00508807

II. This study is registered with ClinicalTrials.gov under the following link: https://clinicaltrials.gov/ct2/show/NCT03749447

III. This study is registered with ClinicalTrials.gov under the following link: https://clinicaltrials.gov/ct2/show/NCT04702997

IV. This study is registered with ClinicalTrials.gov under the following link: https://clinicaltrials.gov/ct2/show/NCT02250781

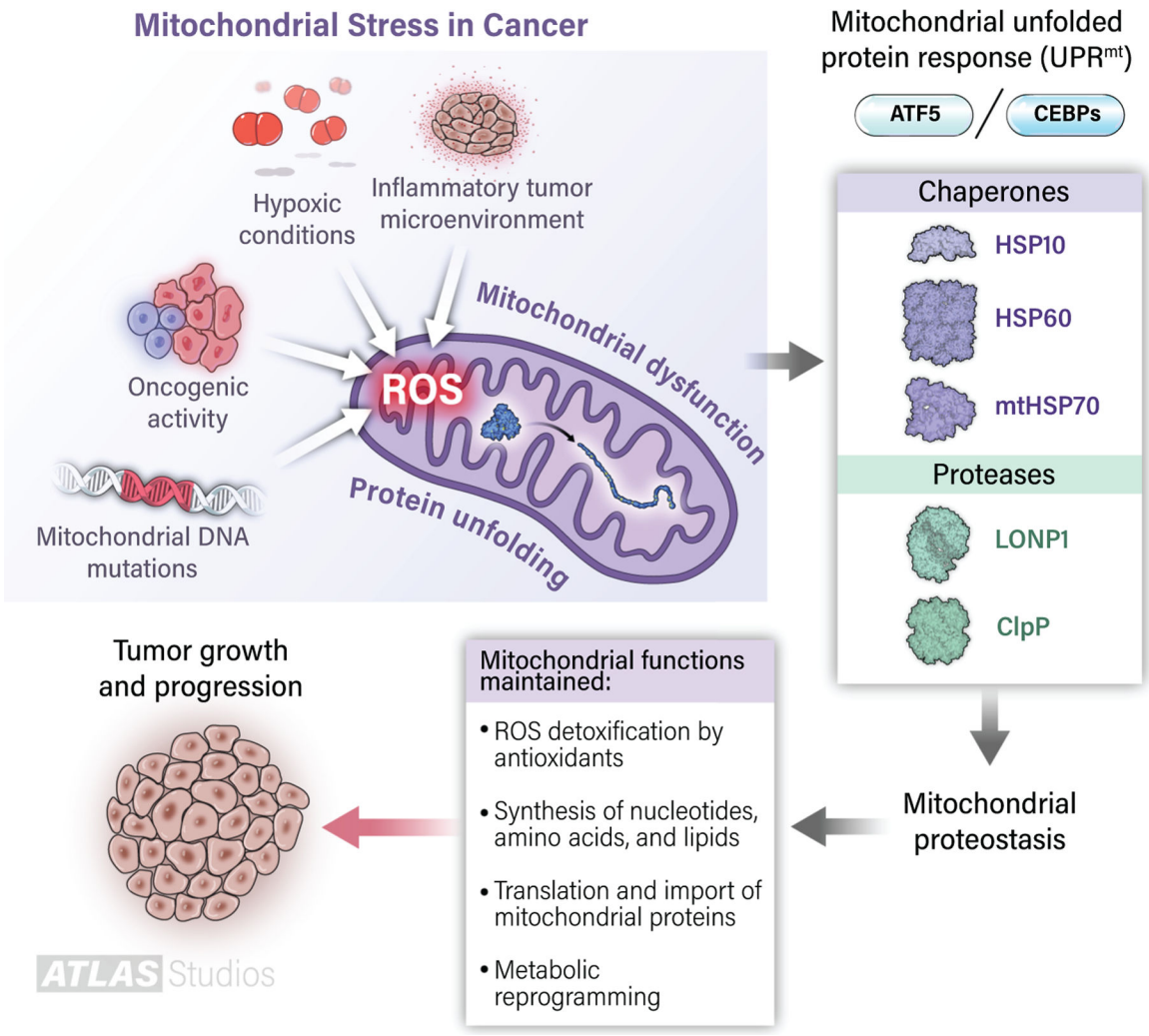

Figure 1: The mitochondrial unfolded protein response (UPR^{mt}) promotes tumor growth and progression.

Various sources of endogenous stress, including genetic alterations, hypoxia, and the presence of inflammatory immune cells, alter mitochondria and enhance ROS production. ROS inflict damage and promote the unfolding/aggregation of mitochondrial proteins. Under these circumstances, the UPR^{mt} activates transcription factors such as ATF5 and CEBPs to induce the upregulation of chaperones (HSP60, HSP10, mtHSP70) and proteases (LONP1, ClpP), which fold proteins into their proper conformation and dispose of excessively damaged proteins, respectively. Together, this stress-responsive system allows mitochondrial proteostasis and maintenance of multiple tumor-supporting activities by mitochondria.

Table 1:

Key cancer therapeutics that target the mitochondrial unfolded protein response (UPR^{mt})

UPR^{mt} Target	**Drug**	**Mechanism**	**Phase**	**References**
HSP60	Epolactaene and derivatives	Inhibits HSP60/HSP10 chaperone activity	Preclinical	[7]
HSP60	Gold (III) porphyrins	Inhibits HSP60/HSP10 chaperone activity	Preclinical	[8]
HSP60	BSP-SCA hybrid analogs	Inhibits HSP60/HSP10 chaperone activity	Preclinical	[9]
mtHSP70	MKT 077	Inhibits interactions between mtHSP70 and Bag co-chaperones	Phase 1	[10]
mtHSP70	JG-231	Inhibits interactions between mtHSP70 and nucleotide exchange factors	Preclinical	[11]
LONP1	CDDO-Me	Inhibits LONP1 protease activity	Phase 1	[12,I*]
ClpP	ONC201	Hyperactivates ClpP protease activity	Phase 1,2	[IV*]
ClpP	A2-32-01	Inhibits ClpP protease activity	Preclinical	[13]
ATF5, CEBPs	(d/n)-ATF5 peptides	Prevents dimerization of transcription factors	Preclinical	[14]

*Indicates references from ClinicalTrials.gov