Autophagy Promotes Tolerance to Hypoxia through Maintenance of Metabolic Homeostasis

Dan Cojocari [1,4], Ravi N Vellanki [1], Marianne Koritzinsky [1,3,5], Bradly G Wouters [1,2,4,5]

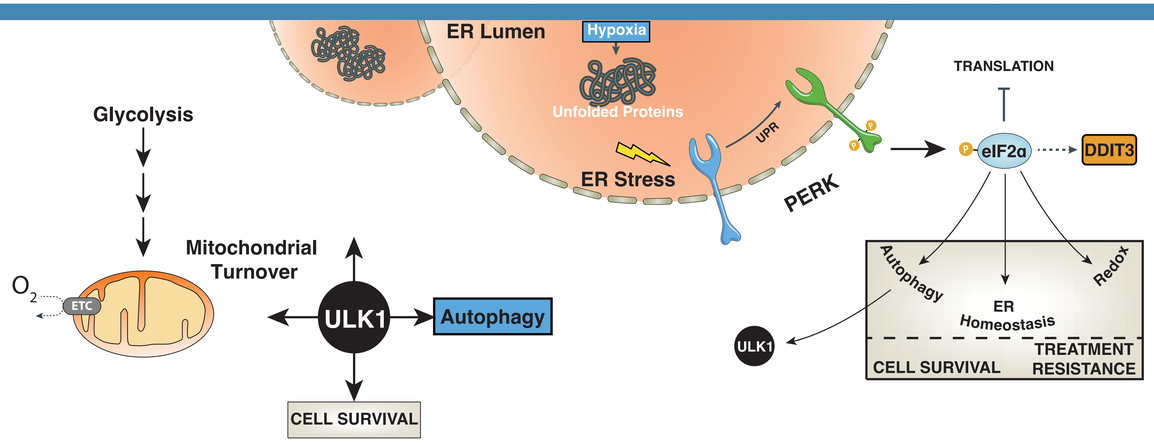
[1] Princess Margaret Cancer Centre, University Health Network, Toronto, Ontario, Canada [2] Ontario Institute for Cancer Research, Toronto, Ontario, Canada.
[3] Institute of Medical Science, [4] Department of Medical Biophysics and [5] Department of Radiation Oncology, University of Toronto, Toronto, Ontario, Canada.

MEDICAL BIOPHYSICS
UNIVERSITY OF TORONTO

UHN Princess Margaret Cancer Centre

Purpose

Oxygenation patterns in solid human tumors are highly heterogeneous both amongst and within tumors, and individual cells can be exposed to mild or extreme hypoxia. Hypoxia is known to influence the behavior of tumor cells in an adverse manner, resulting in poor response to radiotherapy, chemotherapy, and an increased metastatic capacity. The ability of cells to tolerate extreme hypoxia and the unusual metabolic environments found within tumors are not fully understood. Previously we have shown that the PERK-eIF2α arm of UPR contributes to hypoxia tolerance in both cell lines and in human tumor xenografts. Furthermore, we found that during hypoxic exposure signaling through PERK results in increased capacity for autophagy and antioxidant response through direct transcriptional regulation of the autophagy genes and glutathione biosynthesis genes, respectively. We hypothesize that autophagy promotes survival during hypoxia through regulation of metabolic and endoplasmic reticulum (ER) homeostasis.

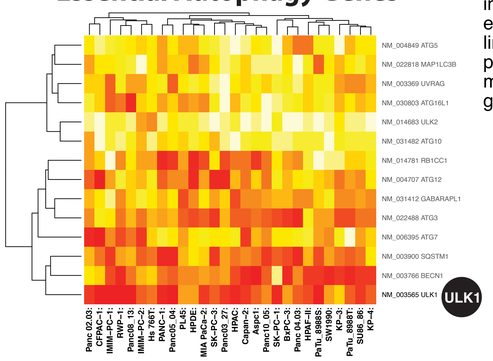


Methods

We used lentiviral-mediated delivery of small hairpin RNA (shRNA) to knockdown genes that regulate autophagy in colon and pancreatic cancer cell lines. Cell proliferation was measured with IncuCyte automated imaging; cell viability using AlamarBlue and clonogenic assays. Mitochondrial levels per cell were quantified using specific dyes followed by flow cytometry. The cells were exposed to hypoxia in HypOxystation by Don Whitley Scientific: H35 for 0.2% and H85 for anoxia <0.02%.

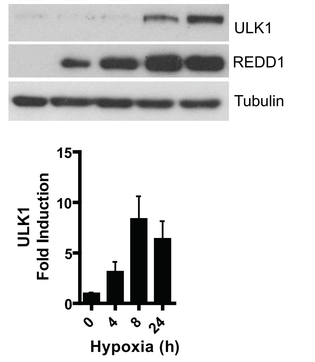
1 ULK1 is a PERK target gene.

A



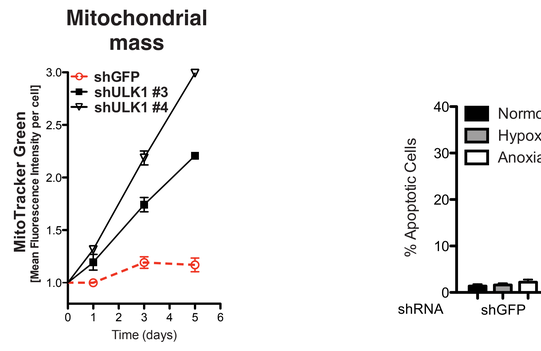
Clustering of the p-values indicates genes that are essential across groups of lines (darker: lower p-value). ULK1 was the most important autophagy gene in this panel.

B



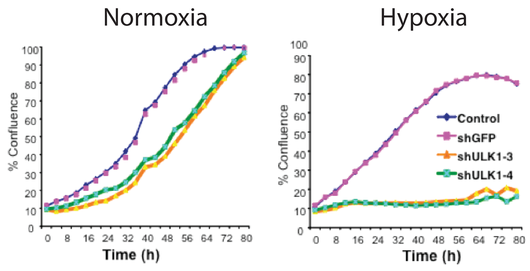
Hypoxia induced expression of ULK1, protein by western blot and transcript levels quantified by qPCR. REDD1 is a known hypoxia induced protein.

2 ULK1 causes accumulation of mitochondria and apoptosis.

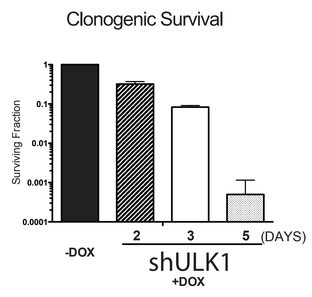


Mitochondrial mass is significantly increased in HCT116 and KP4 cells following ULK1 knockdown.
Levels of apoptosis are significantly increased in the knockdown cells exposed to hypoxia for 24h.

3 ULK1 knockdown causes progressive cell damage and sensitization to hypoxia.

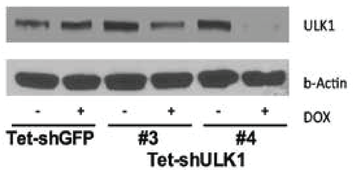


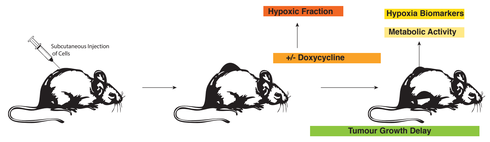
ULK1 knockdown with two individual shRNA's specifically sensitizes cells to hypoxia (0.2%) as measured by cell proliferation.



ULK1 knockdown with DOX inducible shRNA's specifically sensitizes cells over a time period of 5 days.

4 inducible inhibition of ULK1 for *in vivo* experiments.





Validated shRNA for ULK1 were cloned into the Tet-ON pLKO inducible shRNA lentiviral system and stably expressed in HCT116 cells. ULK1 protein is significantly knocked down after 5 day exposure to doxycycline with the two shRNAs.

Conclusions

Together our data suggest that ULK1 and autophagy promote hypoxia tolerance through two distinct mechanisms:

Upon ULK1 knockdown, loss of mitochondrial homeostasis leads to enhanced levels of hypoxia and cell death.

These new mechanistic understanding of the importance of autophagy in metabolism and cell survival provides new opportunities for development of hypoxia directed therapies.

Ontario
CIHR IRSC Canadian Institutes of Health Research / Instituts de recherche en santé du Canada
HP5
The Terry Fox Research Institute
L'Institut de recherche Terry Fox