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Targeting Mitochondria
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GLUTAMINE DEPRIVATION INDUCED SENSITIZATION OF CANCER CELL INHIBITION BY LOW-DOSE MITOCHONDRIAL ETC COMPLEX I INHIBITOR: A LABEL-FREE QUANTIFICATION APPROACH TO STUDY ALTERATION IN MITOCHONDRIAL PROTEOME

Abhinav Prasad, Ashim Chandra Roy, Ilora Ghosh* (iloraghosh17@gmail.com)
Biochemistry and Environmental Toxicology Laboratory, Lab # 103, School of Environmental Sciences, Jawaharlal Nehru University, New Delhi-110067, INDIA

ABSTRACT

Introduction: Human cervical cancer cells HeLa exhibit "glutamine (Q) addiction" for its growth and proliferation. We adopted a potential antitumor strategy comprising exposure of HeLa cells to low-dose of mitochondrial (mt) complex I inhibitor, rotenone (R) in Q deprived growth condition (Q-/R) for 24 h and delineated probable underlying mechanisms.

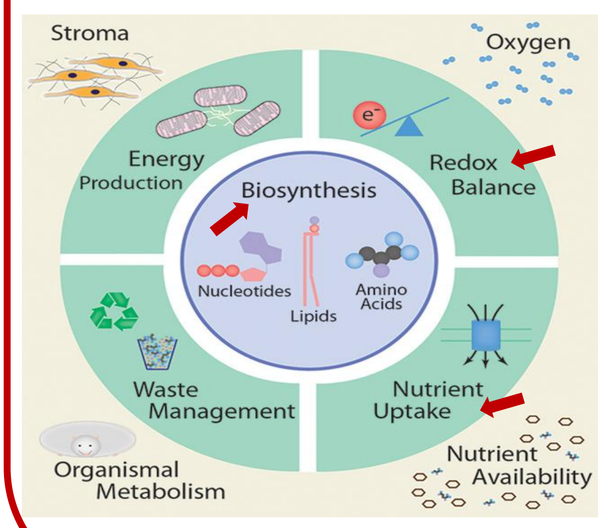
Materials & Methods: MTT, clonogenic & cell migration assays, Flow cytometry, LC-MS/MS-based label-free quantitative (LFQ) proteomics and Bioinformatic analysis

Results: Q-/R significantly reduced cell viability and compromised mitochondrial health due to loss of $\Delta\psi_{m,}$ increased mt ROS and decreased mt mass. S-phase cell cycle arrest and cell migration inhibition were also confirmed. Mitochondrial proteome profiling identified 147 mt proteins with 46 differentially expressed proteins (DEPs), including 19 significantly up-regulated and 27 down-regulated. Functional enrichment analysis of DEPs revealed that the inhibition of tumorigenesis involved alterations in key KEGG pathways such as TCA cycle, Oxidative phosphorylation, Chemical carcinogenesis- reactive oxygen species, Fatty acid degradation, Biosynthesis of amino acid and Pyruvate metabolism.

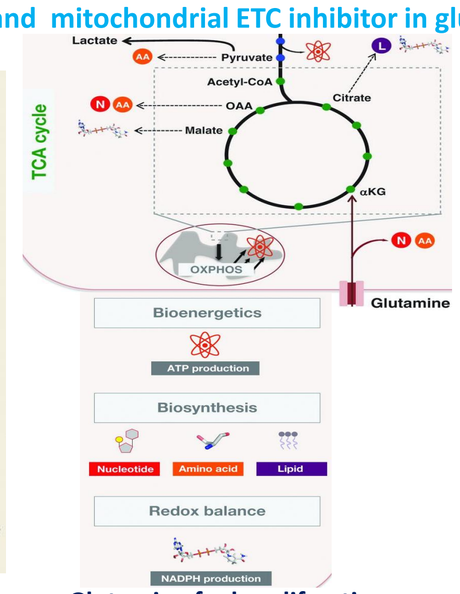
Conclusion: This study provided detailed insight into the proposed anticancer strategy targeting mitochondrial complex I and cancer cell dependency on extracellular glutamine. However, its potential to aid in adopting new strategies to better control proliferation of other cancer cell type needs to be further examined in other pre-clinical and animal models.

INTRODUCTION

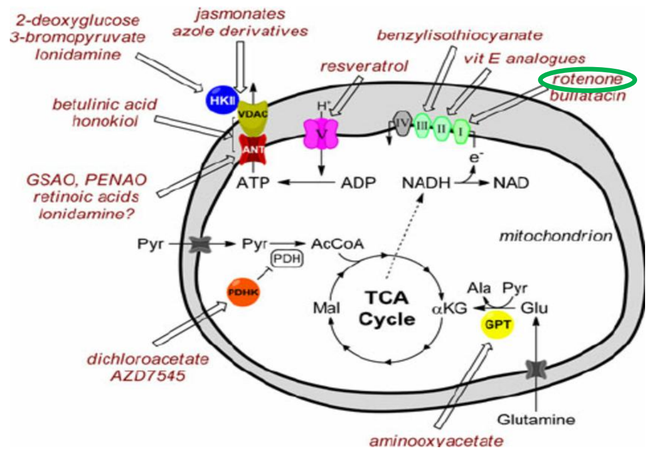
Targeting alterations in nutrient availability (uptake), macromolecular biosynthesis and redox homeostasis by glutamine deprivation and mitochondrial ETC inhibitor in glutamine addicted cancer cells



Metabolic reprogramming in cancer cells

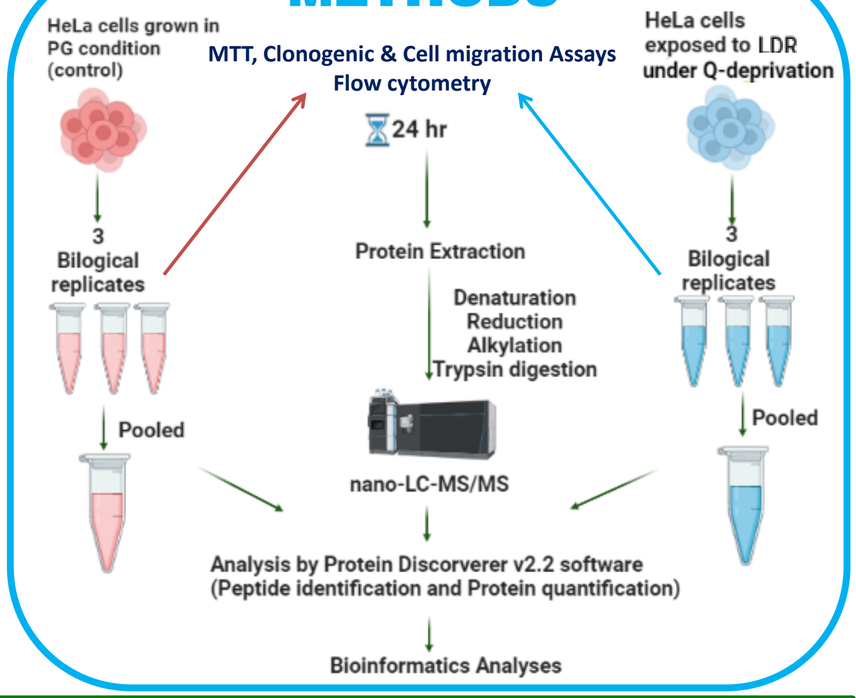


Glutamine fuel proliferation
(Cantor, J. R. et al. *Cancer Discov.* 2012; **2**: 881-898)



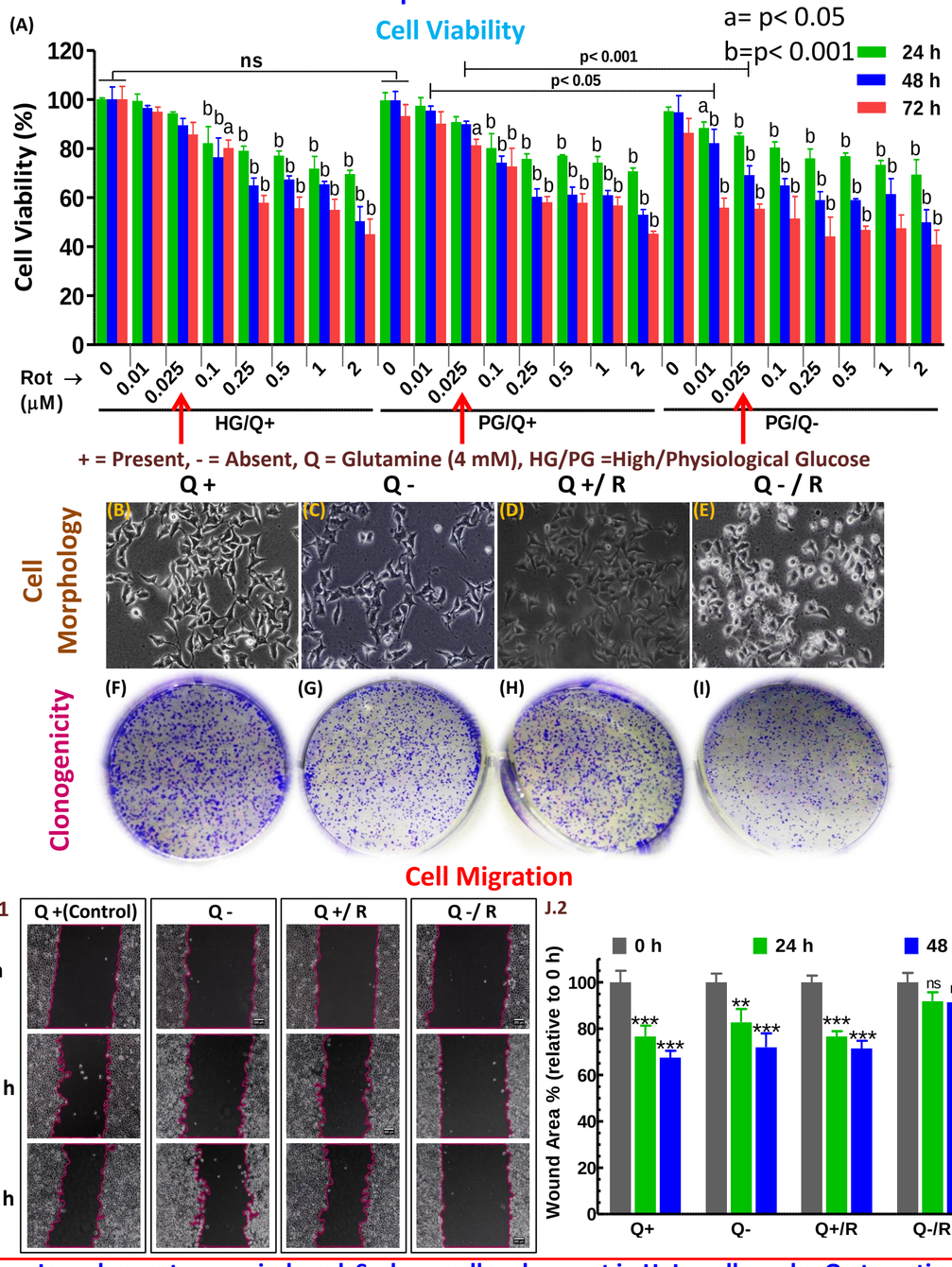
Mitochondrial metabolism inhibitors as anticancer agents
(Ramsay, E.E. et al. *Pharm Res*. 2011; **28**:2731)

METHODS

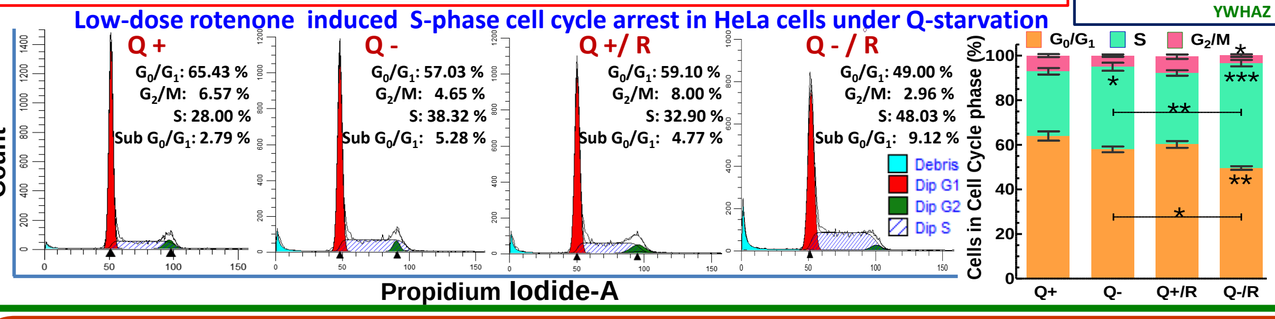


RESULTS

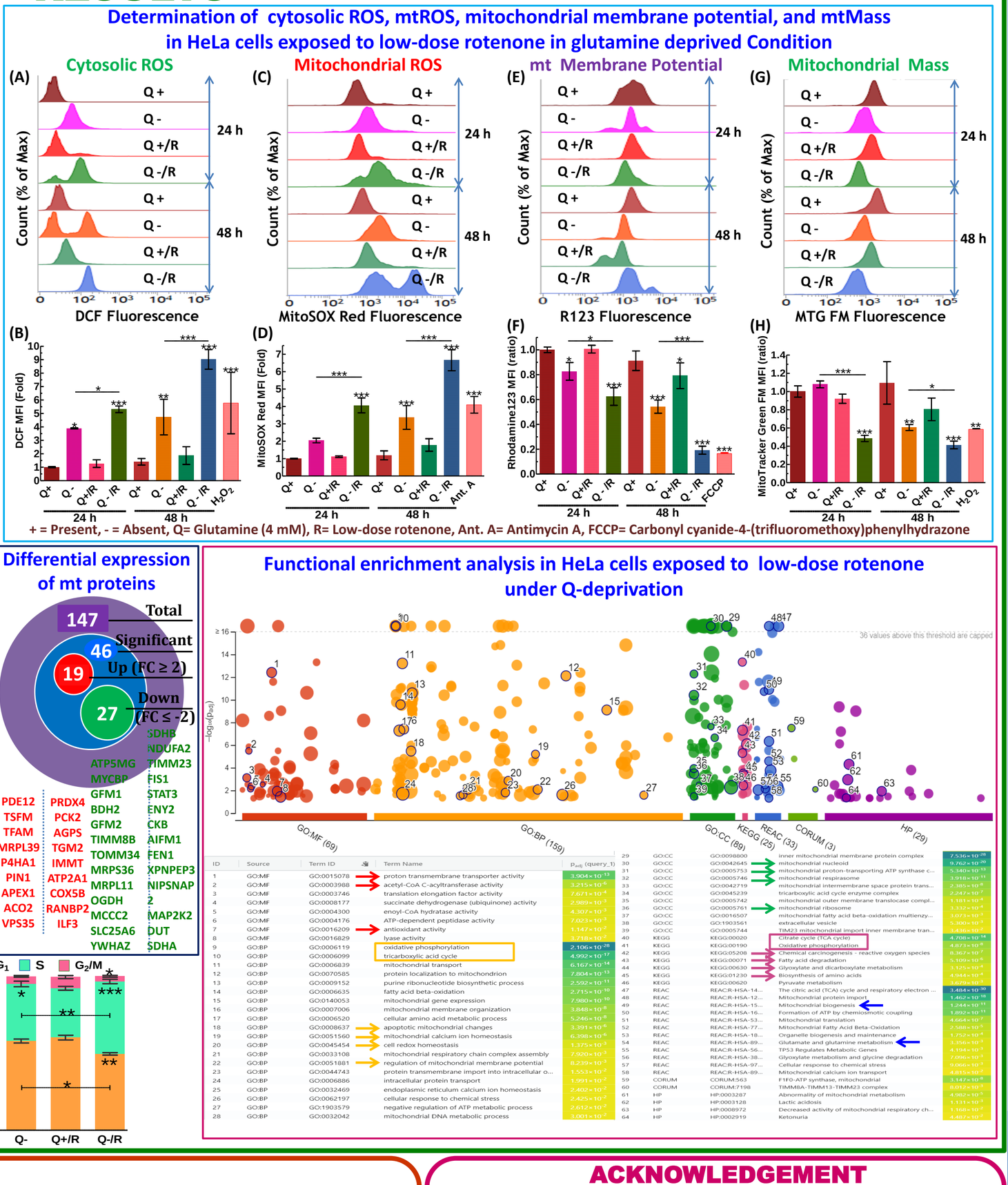
Glutamine deprivation sensitized HeLa cells to cell death & cell migration inhibition after exposure to low-dose rotenone



Low-dose rotenone induced S-phase cell cycle arrest in HeLa cells under Q-starvation



Determination of cytosolic ROS, mtROS, mitochondrial membrane potential, and mtMass in HeLa cells exposed to low-dose rotenone in glutamine deprived Condition



Differential expression of mt proteins

Functional enrichment analysis in HeLa cells exposed to low-dose rotenone under Q-deprivation

ID	Source	Term ID	Term Name	P_{adj} (query_1)
1	GO:MF	GO:0015078	proton transmembrane transporter activity	3.904×10^{-13}
2	GO:MF	GO:0003988	acetyl-CoA C-acyltransferase activity	3.215×10^{-6}
3	GO:MF	GO:0003746	translation elongation factor activity	7.671×10^{-4}
4	GO:MF	GO:0008177	succinate dehydrogenase (ubiquinone) activity	2.989×10^{-3}
5	GO:MF	GO:0004300	enoyl-CoA hydratase activity	4.307×10^{-3}
6	GO:MF	GO:0004176	ATP-dependent peptidase activity	7.623×10^{-3}
7	GO:MF	GO:0016209	antioxidant activity	1.147×10^{-2}
8	GO:MF	GO:0016829	lyase activity	3.718×10^{-2}
9	GO:BP	GO:0006119	oxidative phosphorylation	2.106×10^{-28}
10	GO:BP	GO:0006099	tricarboxylic acid cycle	4.992×10^{-17}
11	GO:BP	GO:0006839	mitochondrial transport	6.167×10^{-14}
12	GO:BP	GO:0070585	protein localization to mitochondrion	7.804×10^{-13}
13	GO:BP	GO:0009152	purine ribonucleotide biosynthetic process	2.592×10^{-11}
14	GO:BP	GO:0006635	fatty acid beta-oxidation	2.715×10^{-10}
15	GO:BP	GO:0140053	mitochondrial gene expression	7.980×10^{-10}
16	GO:BP	GO:0007006	mitochondrial membrane organization	3.848×10^{-8}
17	GO:BP	GO:0006520	cellular amino acid metabolic process	5.246×10^{-8}
18	GO:BP	GO:0008637	apoptotic mitochondrial changes	3.391×10^{-6}
19	GO:BP	GO:0051560	mitochondrial calcium ion homeostasis	6.398×10^{-6}
20	GO:BP	GO:0045454	cell redox homeostasis	1.375×10^{-3}
21	GO:BP	GO:0033108	mitochondrial respiratory chain complex assembly	7.920×10^{-3}
22	GO:BP	GO:0051881	regulation of mitochondrial membrane potential	8.239×10^{-3}
23	GO:BP	GO:0044743	protein transmembrane import into intracellular o...	1.553×10^{-2}
24	GO:BP	GO:0006886	intracellular protein transport	1.991×10^{-2}
25	GO:BP	GO:0032469	endoplasmic reticulum calcium ion homeostasis	2.402×10^{-2}
26	GO:BP	GO:0062197	cellular response to chemical stress	2.425×10^{-2}
27	GO:BP	GO:1903579	negative regulation of ATP metabolic process	2.612×10^{-2}
28	GO:BP	GO:0032042	mitochondrial DNA metabolic process	3.001×10^{-2}
29	GO:CC	GO:0098800	inner mitochondrial membrane protein complex	7.536×10^{-28}
30	GO:CC	GO:0042645	mitochondrial nucleoid	9.762×10^{-20}
31	GO:CC	GO:0005753	mitochondrial proton-transporting ATP synthase c...	5.380×10^{-13}
32	GO:CC	GO:0005746	mitochondrial respirasome	3.918×10^{-11}
33	GO:CC	GO:0042719	mitochondrial intermembrane space protein trans...	2.385×10^{-8}
34	GO:CC	GO:0045239	tricarboxylic acid cycle enzyme complex	2.247×10^{-7}
35	GO:CC	GO:0005742	mitochondrial outer membrane translocase compl...	1.181×10^{-4}
36	GO:CC	GO:0005761	mitochondrial ribosome	1.332×10^{-4}
37	GO:CC	GO:0016507	mitochondrial fatty acid beta-oxidation multienzy...	3.073×10^{-3}
38	GO:CC	GO:1903561	extracellular vesicle	5.300×10^{-3}
39	GO:CC	GO:0005744	TIM23 mitochondrial import inner membrane tran...	5.486×10^{-3}
40	KEGG	KEGG:00020	Citrate cycle (TCA cycle)	4.708×10^{-14}
41	KEGG	KEGG:00190	Oxidative phosphorylation	4.873×10^{-8}
42	KEGG	KEGG:05208	Chemical carcinogenesis - reactive oxygen species	8.367×10^{-7}
43	KEGG	KEGG:00071	Fatty acid degradation	5.109×10^{-6}
44	KEGG	KEGG:00630	Glyoxylate and dicarboxylate metabolism	3.125×10^{-4}
45	KEGG	KEGG:01230	Biosynthesis of amino acids	4.944×10^{-4}
46	KEGG	KEGG:00620	Pyruvate metabolism	1.679×10^{-3}
47	REAC	REAC:R-HSA-14...	The citric acid (TCA) cycle and respiratory electron ...	3.484×10^{-30}
48	REAC	REAC:R-HSA-12...	Mitochondrial protein import	5.462×10^{-18}
49	REAC	REAC:R-HSA-15...	Mitochondrial biogenesis	1.244×10^{-11}
50	REAC	REAC:R-HSA-16...	Formation of ATP by chemiosmotic coupling	1.892×10^{-11}
51	REAC	REAC:R-HSA-53...	Mitochondrial translation	4.664×10^{-7}
52	REAC	REAC:R-HSA-77...	Mitochondrial Fatty Acid Beta-Oxidation	2.988×10^{-5}
53	REAC	REAC:R-HSA-18...	Organelle biogenesis and maintenance	1.752×10^{-4}
54	REAC	REAC:R-HSA-89...	Glutamate and glutamine metabolism	1.356×10^{-3}
55	REAC	REAC:R-HSA-56...	TP53 Regulates Metabolic Genes	4.194×10^{-3}
56	REAC	REAC:R-HSA-38...	Glyoxylate metabolism and glycine degradation	7.096×10^{-3}
57	REAC	REAC:R-HSA-97...	Cellular response to chemical stress	9.066×10^{-3}
58	REAC	REAC:R-HSA-89...	Mitochondrial calcium ion transport	4.815×10^{-2}
59	CORUM	CORUM:563	F1F0-ATP synthase, mitochondrial	3.147×10^{-8}
60	CORUM	CORUM:7198	TIMM8A-TIMM13-TIMM23 complex	8.012×10^{-3}
61	HP	HP:0003287	Abnormality of mitochondrial metabolism	4.982×10^{-5}
62	HP	HP:0003128	Lactic acidosis	1.131×10^{-3}
63	HP	HP:0008972	Decreased activity of mitochondrial respiratory ch...	1.168×10^{-2}
64	HP	HP:0002919	Ketonuria	4.467×10^{-2}

CONCLUSION

- Q- deprivation for 24 h sensitized HeLa cells to growth inhibition by Low-dose (25 nM) of rotenone, the dose that was otherwise non-toxic in normal (PG) media
- The increased anticancer effect was due to cyto-mt ROS-induced oxidative stress and loss of mitochondrial function due to drop in $\Delta\psi_m$, excess mt superoxide, and a decreased mt mass.
- Growth and proliferation inhibitory effects on HeLa cells were established by in the S-phase cell cycle arrest and halt in cell migration
- Functional enrichment analysis of DEPs revealed that the inhibition of tumorigenesis involved alterations in key KEGG pathways such as TCA cycle, Oxidative phosphorylation, Chemical carcinogenesis- reactive oxygen species, Fatty acid degradation, Glyoxylate and dicarboxylate metabolism, Biosynthesis of amino acid and Pyruvate metabolism.
- After analysing its molecular principles, efficacy, and avoiding safety concerns through animal model study and clinical trials, this research may add to our understanding of nutritionally based anticancer signalling in cervical and other cancers.

ACKNOWLEDGEMENT

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