Advancing Drug Discovery for Neurodegenerative Diseases: A High-Throughput Screening Pipeline Targeting Mitochondrial Dysfunction in Patient-Derived Neurons

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Abstract

Mitochondrial dysfunction is a common underlying phenotype observed in various neurodegenerative diseases. Inefficient mitochondria with decreased ATP production have been linked with neurodegenerative diseases. Therefore, targeting mitochondrial dysfunction and energy production holds potential in finding effective treatments for neurodegenerative diseases, which currently lack pharmaceutical treatments. In this poster, We will discuss the development of a high throughput screening method capable of quantifying the level of mitochondrial dysfunction in cells and assessing the impact of potential drug treatments according to mechanisms based on dysfunctional mitochondria respiration and pH changes in a buffer-less medium. This study utilizes cell strain mtDNA 8993, which effectively mimics the phenotype observed in mitochondrial dysfunction associated with neurodegenerative diseases.

Figure 1: Mitochondria Dysfunction

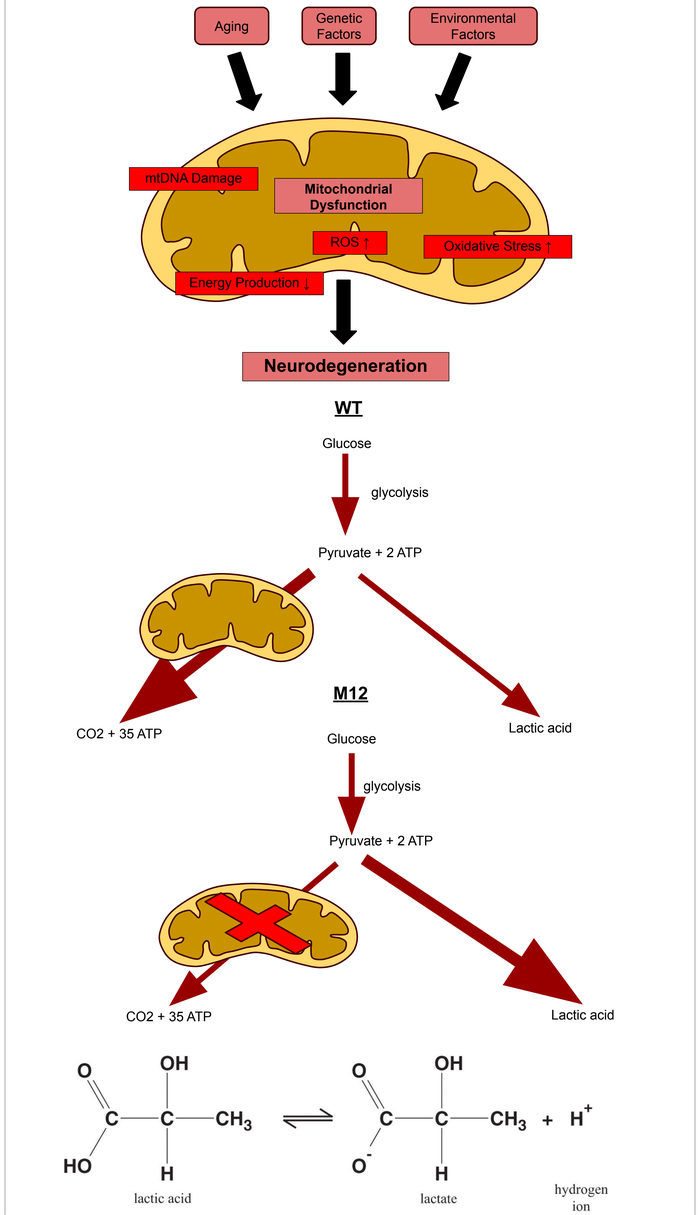


Figure 2: mtDNA mutation T8993G in MT-ATP6

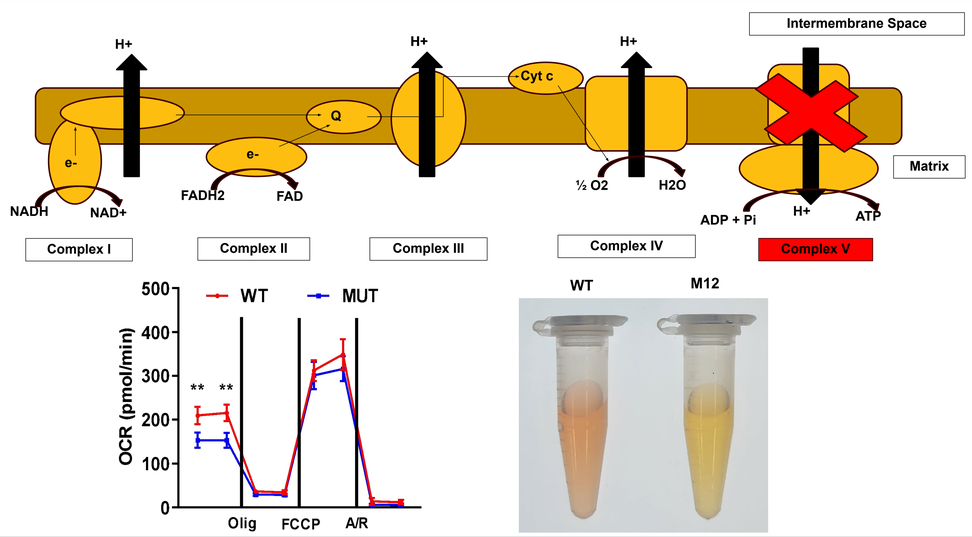
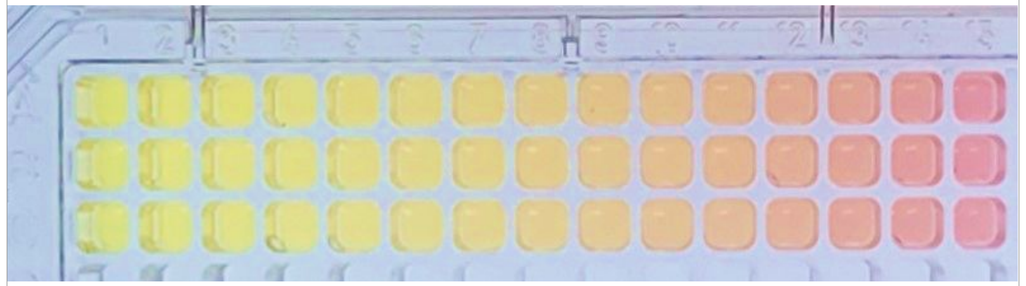
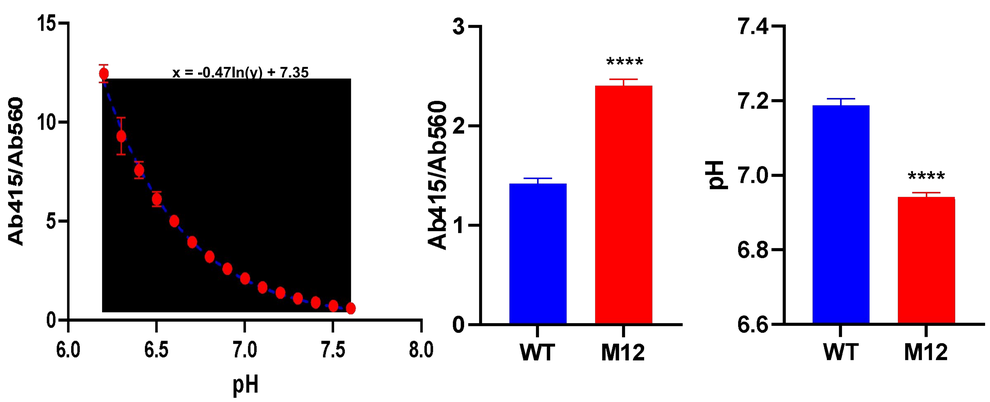


Figure 3: Mitochondrial Dysfunction v. pH

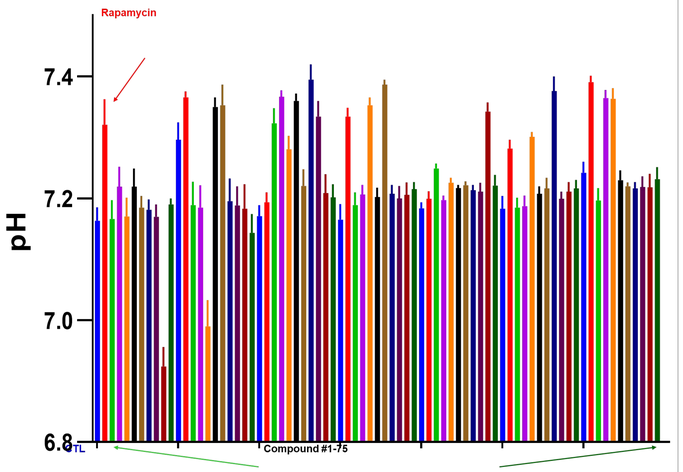


pH:	6.2	6.3	6.4	6.5	6.6	6.7	6.8	6.9	7	7.1	7.2	7.3	7.4	7.5	7.6
Ab415 /Ab560	12.30	8.77	7.36	5.92	4.88	3.83	3.15	2.54	2.10	1.65	1.40	1.11	0.90	0.73	0.60
	12.97	10.39	8.07	6.55	5.25	4.19	3.38	2.70	2.17	1.69	1.40	1.12	0.91	0.74	0.59
	12.13	8.77	7.34	5.92	4.93	3.84	3.12	2.57	2.08	1.65	1.39	1.11	0.91	0.74	0.61



Mitochondrial Dysfunction v. pH

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	1.36	1.33	1.33	1.49	1.48	1.36	1.33	1.43	1.38	1.30	1.23	1.44	1.51	1.42	1.40	1.32	1.33	1.32	1.32	1.37	1.42	1.39	1.39	1.44
B	1.37	1.55	1.54	1.20	0.98	1.59	1.53	1.45	1.31	1.52	1.57	1.43	1.34	1.49	1.44	1.47	1.47	1.52	1.52	2.60	2.62	1.44	1.41	1.43
C	1.37	1.43	1.44	1.06	1.03	1.40	1.41	1.26	1.27	1.39	1.39	1.26	1.26	1.39	1.37	1.38	1.41	1.43	1.41	2.41	2.29	1.38	1.40	1.39
D	1.34	1.16	1.19	0.97	0.99	1.43	1.56	1.48	1.55	2.31	2.33	1.02	1.03	1.09	1.00	1.45	1.51	1.48	1.50	1.54	1.52	1.67	1.60	1.38
E	1.43	1.09	1.05	0.96	0.95	1.36	1.30	1.33	1.34	2.02	1.97	0.98	0.97	0.95	0.94	1.30	1.31	1.33	1.34	1.34	1.32	1.49	1.46	1.39
F	1.40	1.48	1.52	1.40	1.45	1.10	1.10	0.95	0.95	1.19	1.21	0.98	0.99	1.35	1.40	0.91	0.97	1.06	1.09	1.43	1.41	1.40	1.44	1.32
G	1.45	1.46	1.40	1.38	1.35	1.05	0.99	0.98	0.98	1.14	1.10	0.95	1.00	1.30	1.23	0.90	0.86	1.02	0.97	1.32	1.25	1.34	1.31	1.31
H	1.44	1.52	1.55	1.06	1.06	1.42	1.48	1.40	1.38	1.02	1.01	1.41	1.39	0.94	0.93	1.37	1.40	1.42	1.43	1.42	1.39	1.36	1.35	1.28
I	1.34	1.49	1.38	1.01	1.01	1.40	1.34	1.34	1.31	0.97	0.98	1.36	1.32	0.92	0.91	1.33	1.32	1.34	1.32	1.32	1.31	1.30	1.32	1.45
J	1.32	1.42	1.46	1.35	1.42	1.22	1.26	1.40	1.37	1.30	1.32	1.34	1.32	1.33	1.32	1.34	1.35	1.39	1.36	1.05	1.03	1.37	1.34	1.44
K	1.33	1.40	1.42	1.36	1.38	1.24	1.24	1.37	1.40	1.28	1.31	1.33	1.32	1.30	1.31	1.31	1.35	1.32	1.31	0.98	1.01	1.27	1.29	1.36
L	1.31	1.45	1.50	1.18	1.19	1.44	1.46	1.45	1.46	1.12	1.12	1.37	1.38	1.39	1.33	1.00	0.97	1.41	1.39	1.40	1.35	1.38	1.31	1.40
M	1.40	1.39	1.37	1.13	1.13	1.43	1.36	1.37	1.38	1.09	1.11	1.36	1.31	1.32	1.28	0.90	0.92	1.36	1.35	1.32	1.31	1.30	1.33	1.36
N	1.42	1.21	1.31	0.91	0.94	1.31	1.39	0.95	0.98	0.95	0.97	1.31	1.34	1.32	1.34	1.30	1.36	1.35	1.37	1.38	1.37	1.32	1.34	1.29
O	1.40	1.28	1.24	0.90	0.92	1.43	1.42	0.95	1.00	0.95	1.02	1.25	1.27	1.31	1.31	1.32	1.34	1.28	1.29	1.27	1.28	1.24	1.25	1.33
P	1.37	1.40	1.41	1.39	1.43	1.34	1.32	1.36	1.37	1.51	1.46	1.39	1.40	1.39	1.42	1.27	1.30	1.39	1.38	1.36	1.38	1.39	1.35	1.31



Summary

Our research aimed to quantify the phenotype of mitochondrial dysfunction in the MT 8993 cell line. We observed that dysfunctional neurons produce higher levels of lactic acid and consume less oxygen than non-dysfunctional neurons, indicating a shift in energy metabolism associated with mitochondrial impairment, specifically a mutation in complex V.

To quantify the pH difference between dysfunctional and non-dysfunctional neurons, we compared pH versus absorbance in the cell medium and generated a standard curve. By applying this curve to dysfunctional and non-dysfunctional strains cultured for the same duration, we identified that dysfunctional neurons have a significantly more acidic pH compared to non-dysfunctional neurons.

This finding provides insight into the potential use of pH levels as a measure of drug effectiveness in treating dysfunction: a more basic pH level in dysfunctional neurons correlates with a greater efficacy of drug treatments. This observation could have significant implications for developing targeted therapeutic interventions for neurodegenerative disorders and mitochondrial disorders. Further investigations into the underlying mechanisms are warranted to translate these findings into potential clinical applications.

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