Proteomic Profiling and Bioinformatics Analyses of Low-Level Laser-Treated Hypoxic Astrocytes

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Background and Aims: The application of low-level laser therapy (LLLT) in brain ischemia provides clinical significance in stroke management. The aim of this study was to explore molecular signatures underlying the neuroprotective effect of LLLT in hypoxic astrocytes.

Methods:

- Mouse astrocytes under normoxic or hypoxic environment
- Confirm hypoxia-inducible factors expression
- MTT assay to assess cell viability of astrocytes after hypoxia
- LLLT at 4J/cm² for 2 days
- Cell lysates for proteomic profiling using LC-MS/MS analyses
- Differentially expressed (DE) proteins between normoxic, hypoxic and LLLT-treated astrocytes analyzed

Result 1: Confirmation of hypoxic induction of astrocytes

- No significant difference in cell viability by MTT assay
- Increased expression of HIF-2α in hypoxic-treated astrocytes

Result 2: Bioinformatics analyses of DE proteins in hypoxic-treated astrocytes

- 25 DE proteins identified in hypoxic astrocytes
 - Involved in glucose metabolism
 - ERK1/2 as central connecting hub
- Increased HS90B, LDHA and THIO involved in HIF signaling and NRF2-mediated oxidative stress response

Table 2. KEGG pathways by DAVID database analysis

KEGG pathway	P value	Molecules	Fold Enrichment
Glycolysis / Gluconeogenesis	3.73*10 ⁻⁴	ALDOA, ENOA, LDHA, PGAM1	25.99
Biosynthesis of antibiotics	0.011	ALDOA, ENOA, LDHA, PGAM1	8.02
Biosynthesis of amino acids	0.012	ALDOA, ENOA, PGAM1	16.50
Glucagon signaling pathway	0.020	LDHA, CALM, PGAM1	12.87
Carbon metabolism	0.026	ALDOA, ENOA, PGAM1	11.09
Spliceosome	0.034	ROA3, HNRPU, IF4A3	9.67

Ingenuity Pathway Analysis

Result 3: Bioinformatics analyses of DE proteins in laser-treated hypoxic astrocytes

- 17 DE proteins identified in laser-treated hypoxic astrocytes
 - 6 proteins involved in post-translational protein regulation through acetylation
- Filamin (FLNA) interact with HS90B, potentially facilitated LLLT-treated hypoxic astrocyte migration

Conclusion: The current findings suggested novel proteins involved in the potential mechanisms of neuroprotective effects exerted by LLLT in brain ischemia. Further *in vitro* validation to confirm the hypothesis is necessary.

Table 1. Top canonical pathways

Canonical pathways	Molecules	p-value
Glycolysis	PGAM1,ALDOA,ENO1	2.96 × 10 ⁻⁶
Gluconeogenesis	PGAM1,ALDOA,ENO1	2.96 × 10 ⁻⁶
EIF2 Signaling	RPL21,EIF3B,EIF4A3	1.76 × 10 ⁻³
Hypoxia Signaling in the Cardiovascular System	LDHA,HSP90AB1	3.05 × 10 ⁻³
Diphthamide Biosynthesis	EEF2	3.29 × 10 ⁻³

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