ALS patient skin derived iAstrocytes predict CuATSM responsiveness

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Background

- Amyotrophic Lateral Sclerosis (ALS) is an adult onset motor neuron disease
- Rate of disease progression is influenced by glial cells, including astrocytes
- Patient diversity and unknown disease cause is a major challenge for drug development and clinical trial design
- Heterogeneity in ALS patients (sALS and fALS) is not reflected in current animal models used to evaluate therapies
  → Direct translation of potential therapeutics using these models is often difficult
- Direct reprogramming of patient cells allows quick generation of disease relevant cell types and facilitates compound testing
  →Our data indicates diverse patient response to therapeutic agents suggesting shared pathways between patient subgroups
- CuATSM is a small molecule drug currently in clinical trials for ALS treatment - it is unclear what subgroup of patients will respond to the drug to date

This study aimed to evaluate whether ALS cell line responders and non-responders to CuATSM treatment could be differentiated in *in vitro* assays. Increased mitochondrial activity was found as the shared parameter unique to responders.

Methods

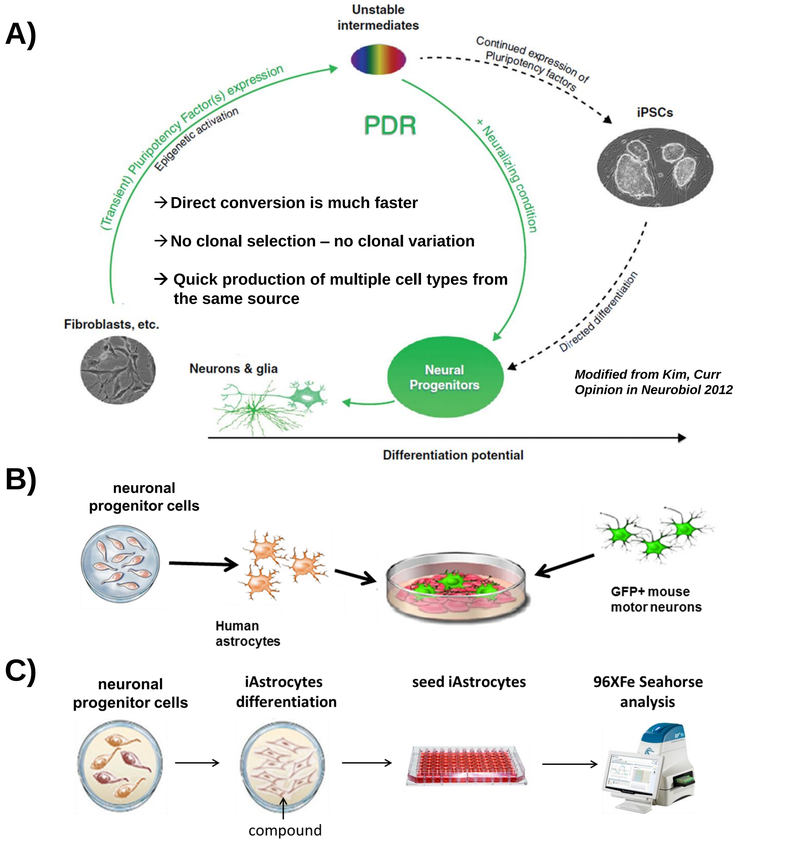

Fig. 1: Direct reprogramming of human skin cells to astrocytes can be used to model ALS. A) Skin fibroblasts are converted to neuronal progenitor cells (Meyer et al, PNAS, 2014) and can then be differentiated into astrocytes (iAstrocytes), neurons and oligodendrocytes. B) Neuronal progenitors are differentiated into iAstrocytes and used for co-culture with GFP+ mouse motor neurons for co-culture survival assays. C) Evaluation of known ALS disease pathways in iAstrocytes using live cell imaging and Seahorse assays.

Results I

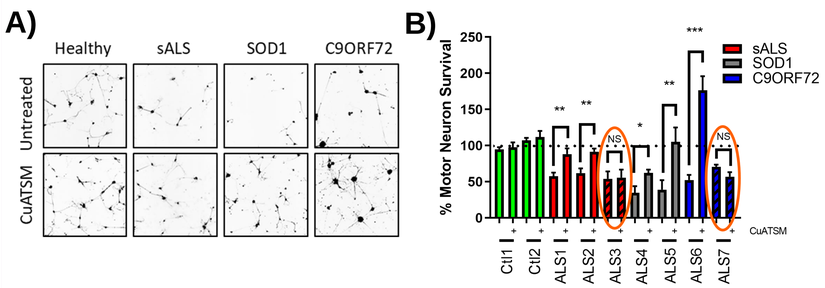

Fig. 2: ALS iAstrocyte mediated toxicity towards motor neurons is rescued by CuATSM treatment in multiple but not all cell lines. A) Representative images of GFP+ motor neurons (shown in black) after 3 days of co-culture on healthy or ALS iastrocytes. B) Quantification of motor neuron survival following co-culture using the INCELL6000 automated imaging system. Data was normalized to average motor neuron survival of healthy controls. Dashed bars (circled) indicate non-responder. Data was analyzed using Student's t-test to compare treated vs. corresponding untreated condition (N=3).

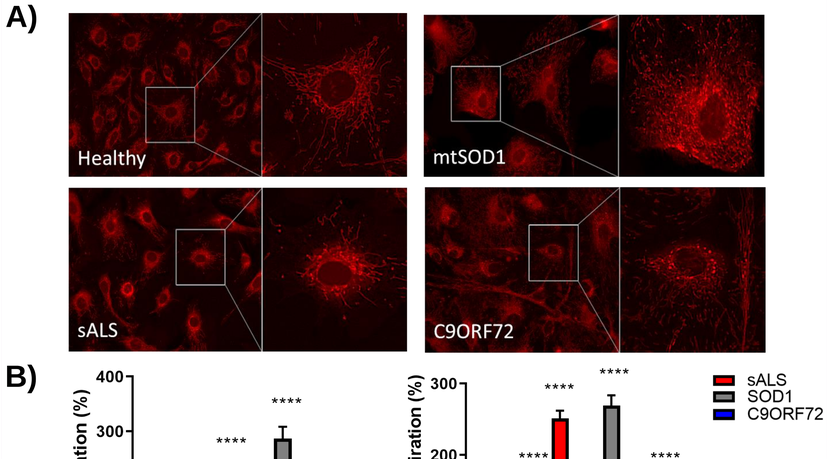

Fig. 3: Correlation between elevated mitochondrial ATP linked respiration and CuATSM responsiveness. A) Representative images of mitochondria stained with MitoTracker Red indicate abnormalities in ALS cell lines. B) Extracellular flux analysis (Seahorse) used to measure basal and ATP-linked respiration in live cells (mitochondrial activity). Dashed bars indicate patient lines classified as non-responders in co-culture assay. Dotted black line represents average control values. CuATSM responding cell lines showed increased mitochondrial activity compared to non-responders. Statistical analysis performed using One-way ANOVA (N=3 by quintuplicate) compared against internal standard line (Ctl1).

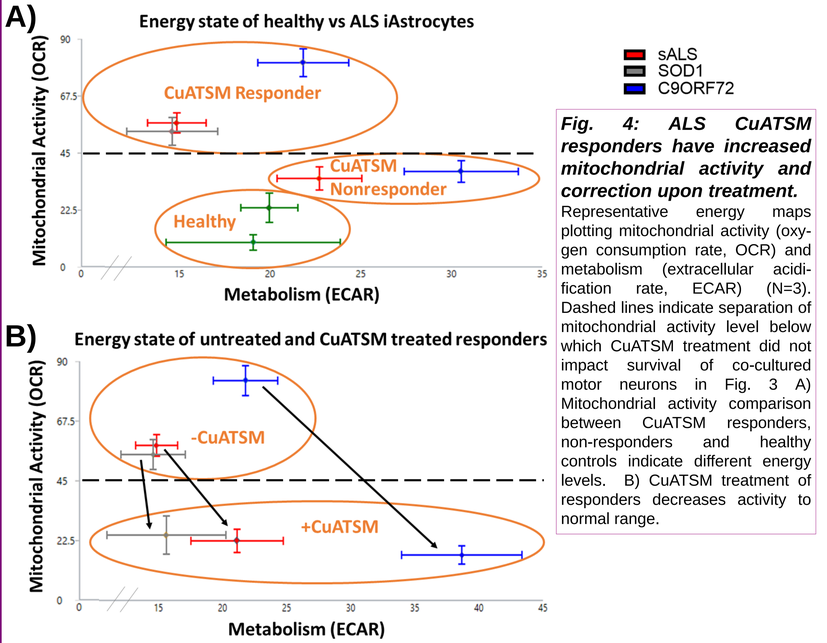

Fig. 4: ALS CuATSM responders have increased mitochondrial activity and correction upon treatment. Representative energy maps plotting mitochondrial activity (oxygen consumption rate, OCR) and metabolism (extracellular acidification rate, ECAR) (N=3). Dashed lines indicate separation of mitochondrial activity level below which CuATSM treatment did not impact survival of co-cultured motor neurons in Fig. 3 A) Mitochondrial activity comparison between CuATSM responders, non-responders and healthy controls indicate different energy levels. B) CuATSM treatment of responders decreases activity to normal range.

Results II

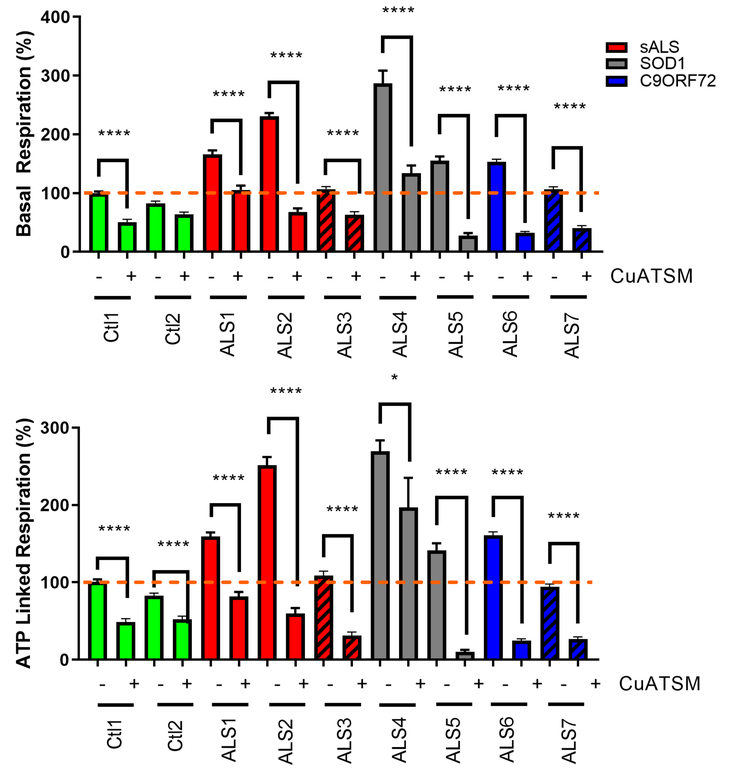

Fig. 5: CuATSM treatment reduces mitochondrial activity to normal levels. Seahorse analyses indicate CuATSM treatment reduces mitochondrial basal and ATP-linked respiration to normal levels. Dashed bars indicate patient lines classified as non-responders in co-culture assay. Dotted orange line represents normal mitochondrial activity range, determined based on co-culture of healthy astrocytes. Statistical analysis preformed using Student's t-test against corresponding untreated condition (N=2-3).

Conclusion

- Rapid reprogramming allows generation of several ALS disease affected cell types including iAstrocytes
  → These cells can be used for evaluation of known disease pathways and for drug screenings
- CuATSM treatment revealed responder and non-responder iAstrocyte cell lines – the ability to respond to CuATSM was correlated with mitochondrial activity

Enhanced understanding of cellular profiles might aid clinicians in determining best treatment approach for patients in upcoming clinical trials.

Next Steps

- Proof system works in clinical applications: can we identify responding and non-responding patients to a certain drug candidate?
- Extensive efforts to expand co-culture system to other neurological and neurodegenerative disorders to create cell line library suitable for extensive drug discovery efforts in neurological and neurodegenerative research field

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