Identification of a novel disease mechanism and development of therapeutics for the recently identified neurodevelopmental disease “NEDAMSS”

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**BACKGROUND**

- The Interferon Regulatory Factor 2 Binding Protein Like (IRF2BPL) is an intronless gene that encodes a member of the IRF2BPL family of transcriptional regulators and is ubiquitously expressed. The function of this protein is unknown.
- Recently, mutations in this gene were found to be associated with NEDAMSS disease:
  - Neurodevelopmental disorder with regression, abnormal movements, loss of speech, and severe in adults and children (Marcogliese et al. 2018).
  - Autosomal dominant inheritance
  - 25 people are known to be affected around the world so far.
- We received four patient fibroblast cell lines having heterozygous nonsense variants in the IRF2BPL gene resulting in the truncation of its RING finger domain (Figure 1).
- In this study, we reprogrammed patient fibroblasts to astrocytes and neurons to understand the disease mechanism and developed promising therapeutic strategies that might be able to slow or halt the progression of the disease.

![Figure 1: Illustration of the different domains in IRF2BPL protein and the location of nonsense variants in the four patient fibroblast cell lines.](image)

**METHODS**

- Cell culture and Western Blot: Patient and healthy fibroblasts were maintained in DMEM media supplemented with 10% FBS. To quantify the expression of IRF2BPL protein in the fibroblasts, Western Blot was conducted using antibody against IRF2BPL (abcam).
- Reprogramming of fibroblasts to astrocytes and neurons:
  - Direct reprogramming of human skin cells can be used to model NEDAMSS. (A) Schematic representation of direct conversion of fibroblasts to neuronal progenitor cells (NPC), which were further differentiated into astrocytes (Meyer et al. 2014). Following conversion, astrocytes were characterized, analyzed by Seahorse assay or co-cultured with stem cell derived GFAP+ motor neurons. (B) Direct chemical reprogramming of fibroblasts to GFAP+ expressing induced neurons using seven small molecules (Yu et al. 2015).

![Figure 2: Direct reprogramming of human skin cells can be used to model NEDAMSS.](image)

**RESULTS**

- IRF2BPL mutations lead to mislocalization of the protein. (A) Immunofluorescence staining shows accumulation of the protein in the cytoplasm of patient astrocytes. (B) Western blot confirms the more accumulation of IRF2BPL in the cytoplasmic extract than in the nucleus. (C) Healthy astrocytes (H1 and H2) infected with lentivirus expressing patient P2 mutant form of IRF2BPL indicates mislocalization and formation of aggregates in the cytoplasm.

![Figure 3: IRF2BPL protein levels is only reduced in one adult patient line.](image)

**CONCLUSION**

- Although Marcogliese et al. suggested a loss of function mechanism for the disease, only adult patient 1911 had significantly reduced IRF2BPL expression in both fibroblasts and astrocytes compared to child patients and healthy controls. This may be explained by the healthy allele compensating the expression of IRF2BPL in younger patients.
- IRF2BPL mutations accumulate in the cytoplasm of astrocytes and could be one cause for neurodegeneration.
- NEDAMSS patient astrocytes were found to have elevated levels of mitochondrial respiration and are toxic to motor neurons in co-culture assays. Treatment with CuATSM significantly improved the respiration levels and motor neuron survival in all patients.
- Development of gene therapy approach using AAV9 as discussed above, to see if it ameliorates the disease phenotype in vitro and potentially in vivo.

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**REFERENCES:**

