REALIZZATO CON IL SOSTEGNO DI

UNIONE EUROPEA
Fondo europeo di sviluppo regionale

Regione Lombardia

fesr

POR FESR 2014-2020 / INNOVAZIONE E COMPETITIVITÀ

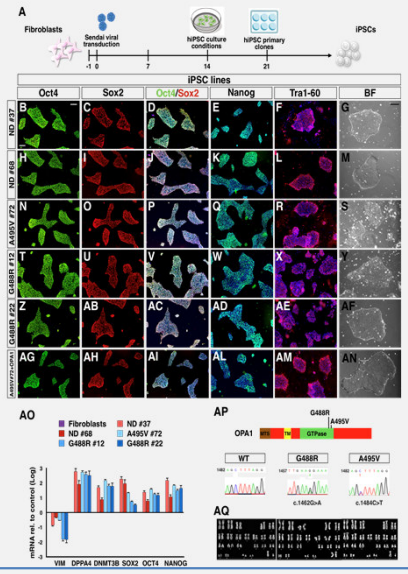
Titolo progetto: Nuovi sistemi biotecnologici iPSC per lo sviluppo farmaceutico nel settore delle malattie neurodegenerative (iPSLight)

Beneficiario: Istituto di Neuroscienze - CNR

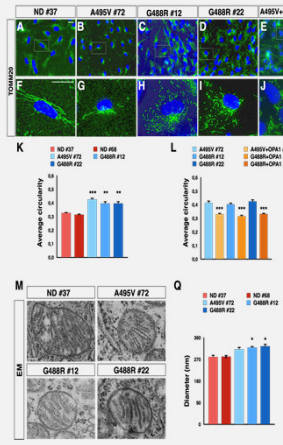
Iniziativa realizzata nell'ambito dell'Asse 1 - Rafforzare la ricerca, lo sviluppo e l'innovazione.

Descrizione progetto: Il progetto **iPSLight** punta allo sviluppo tecnologico di cellule staminali umane riprogrammate iPSC (*induced pluripotent stem cell*) da pazienti con malattie neurodegenerative, applicabili alla ricerca industriale farmaceutica in combinazione con metodiche all'avanguardia di *high-content* e *high-throughput* screening (HCS, HTS).

1.Development and characterization of iPSCs from mutant and normal donor individuals

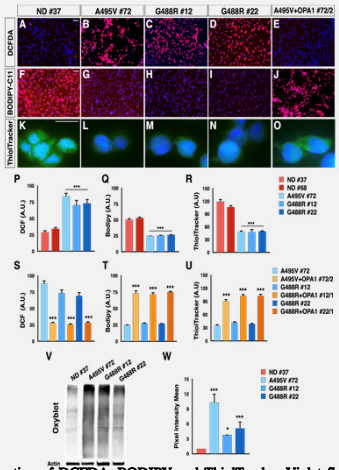


2.OPA1-mutant NPCs exhibit morphological mitochondrial alterations



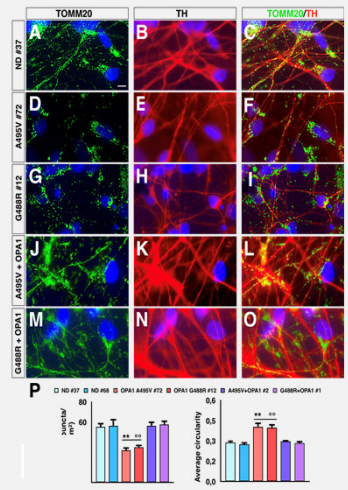
Immunostaining for the protein of the external mitochondrial membrane TOM20 show a filamentous mitochondrial network in ND NPCs, which was significantly fragmented in the PD-OPA1 NPCs. Quantitative measurement of transmission electronic microscopy images confirms that OPA1 mutant mitochondria were reduced in length with an increasing round-shaped morphology.

3.High oxidative stress level in PD-OPA1 NPCs



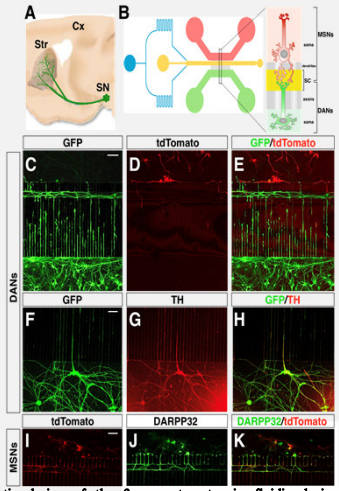
Quantification of DCFDA, BODIPY and ThiolTracker Violet fluorescence evidences a heightened oxidative state in PD-OPA1 NPCs. OPA1 gene correction is effective in rescuing different oxidative state parameters. OxyBlot assay and quantification of carbonylated protein levels confirming the altered oxidative condition of PD-OPA1 NPCs. .

4. PD-OPA1 neurons have an alterate mitochondria distribution



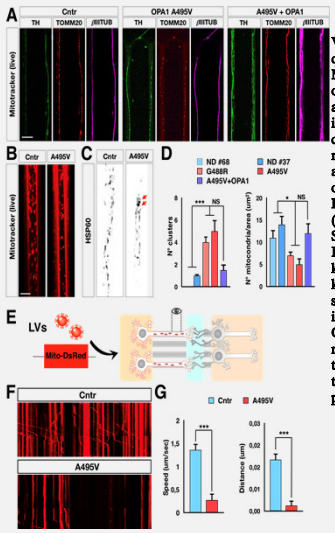
Quantification of TOMM20 stainings shows a reduced mitochondrial numbers and fragmented appearance along the neurites of mutant compared to control and gene-complemented PD-OPA1 neurons.

5. Microfluidic device allow reconstitution of neuronal networks



Schematic design of the 3-compartment microfluidic device with the dopaminergic and striatal lateral chambers connected via microgrooves with the central synaptic channel. Representative images of TH+ dopaminergic neurons and of DARPP32+ medium spiny neurons seeded in the microfluidic device.

6. PD-OPA1 dopaminergic neurons have a reduced number of mitochondria along the axons



Visualization and quantification of MitoTracker orange live signal and HSP60 immunofluorescence confirming the reduction and aberrant clustering of mitochondria in PD-OPA1 axons (arrows in C). (E) Sch. Representative kymographs and kinetics analysis show an impairment of PD-OPA1 mutant mitochondria trafficking along the DA axonal projections.

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