



Doctoral school in «Molecular, cellular and environmental biology»
XLI cycle

**Astrocytes at the crossroads between
neurodegeneration and cancer:
unveiling the pathways for two contrasting fates**

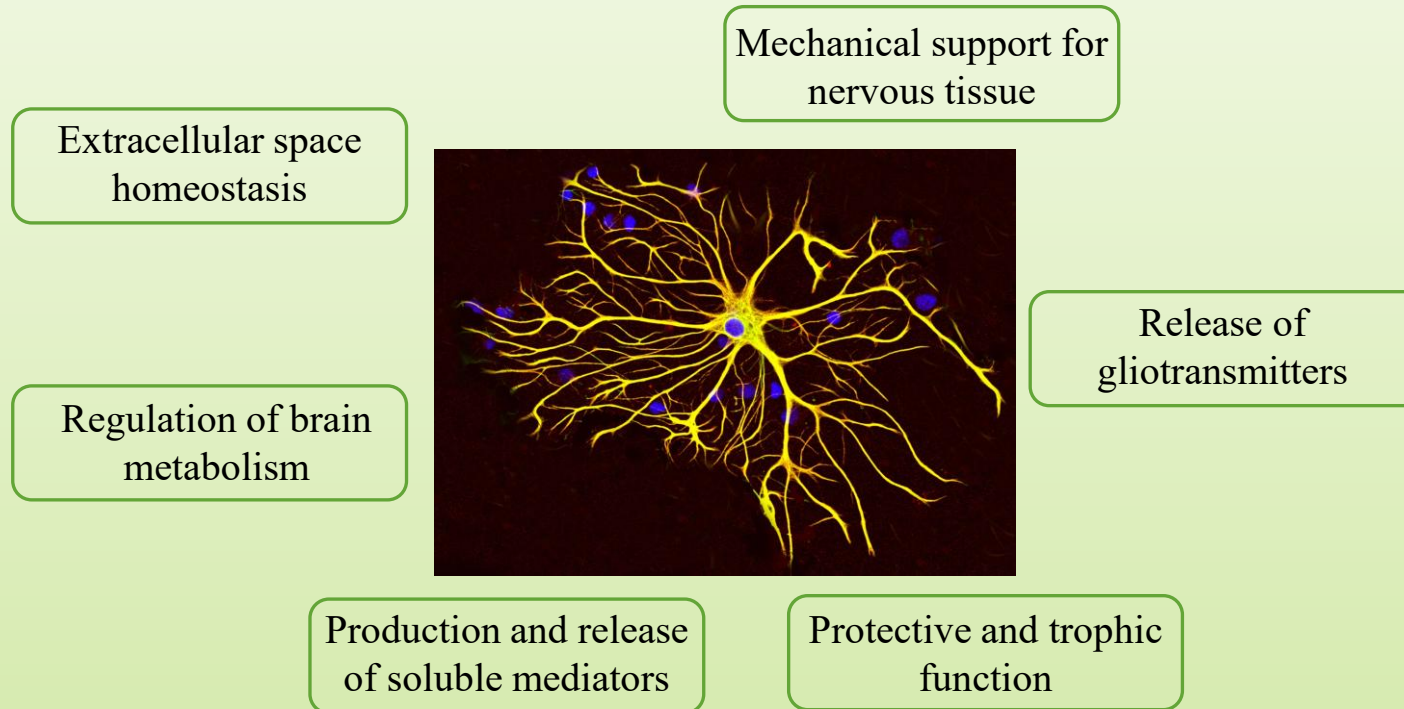
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BACKGROUND

Physiological role of astrocytes

Represent more than 50% of the entire cell population in the Central Nervous System (CNS)



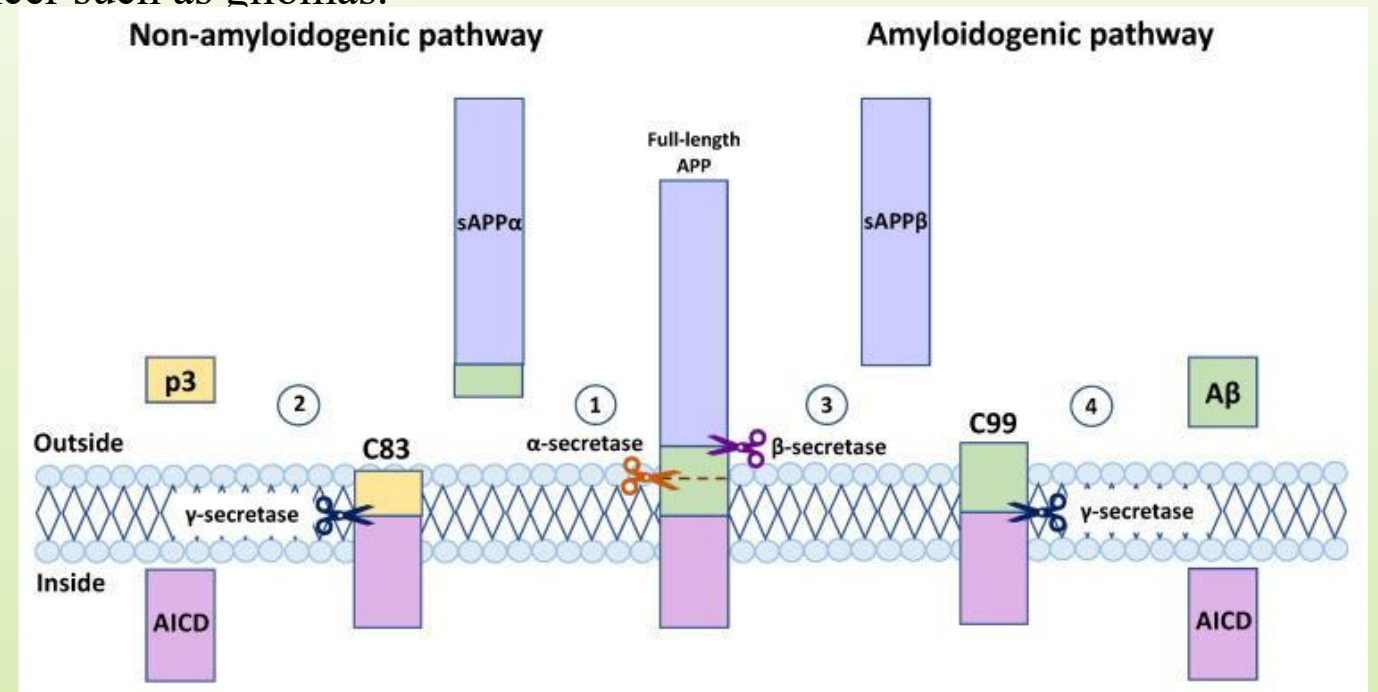
BACKGROUND

Pathologies of CNS:

Neurodegenerative diseases such as Alzheimer's

Cancer such as gliomas.

- Is the most common form of senile dementia
- Histological feature: extracellular plaques of **β -amyloid (β A)** and intracellular neurofibrillary clusters (NFTs) in neurons.



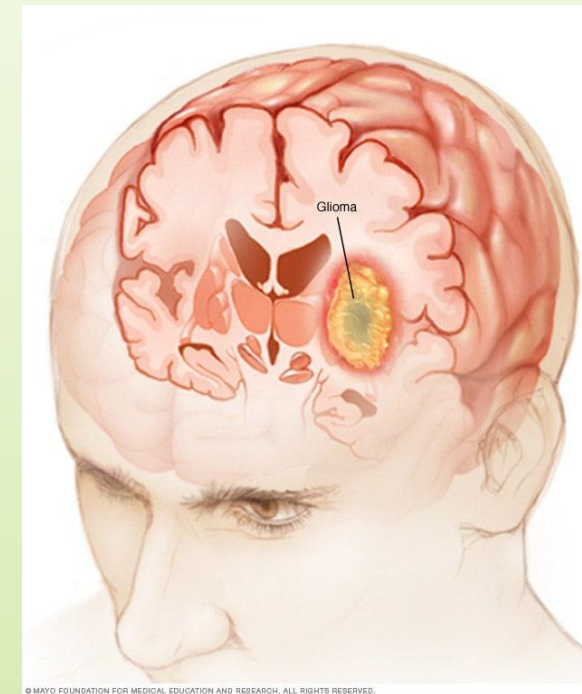
BACKGROUND

Gliomas

~40% of all primary tumors of the CNS

Table 1. Classification of Malignant Gliomas and Survival According to the Surveillance, Epidemiology, and End Results (SEER) Program Registry²

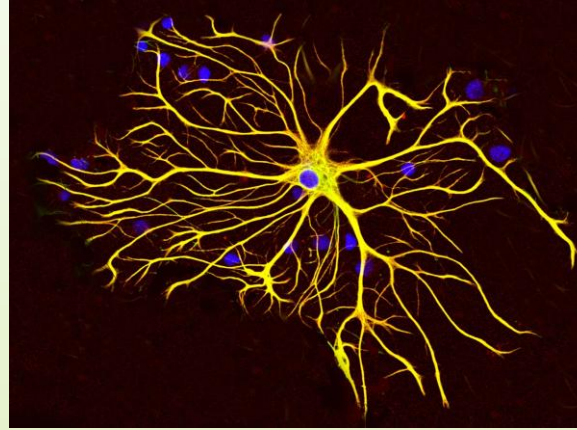
	Survival, %	
	1-Year	5-Year
WHO grade IV tumors ^a		
Glioblastoma and variants ^b	35.7	4.7
WHO grade III tumors ^a		
Anaplastic astrocytoma	60.1	25.9
Anaplastic oligodendroglioma	81	49.4
Anaplastic oligoastrocytomas	NA	NA



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PROJECT AIMS

The pathways for two contrasting fates



Alzheimer's disease

Gliomas

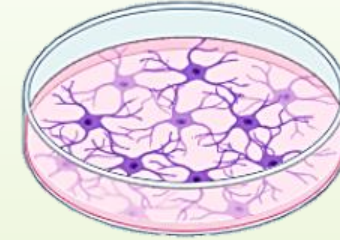
- Antioxidant response
- Iron homeostasis
- Organelles structure and function
- Inter-organelles communication

EXPERIMENTAL MODEL



Alzheimer's disease model

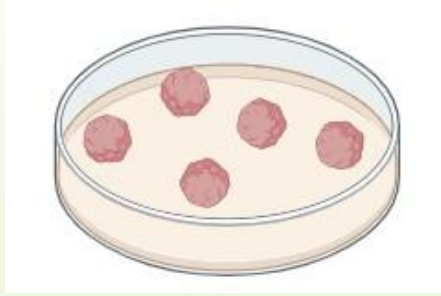
- Primary human astrocytes/
Astrocytes differentiated from iPSC
- Human Astrocytes + β -Amyloid oligomers
- Neurons differentiated from neuroblastoma or iPSC



Gliomas model

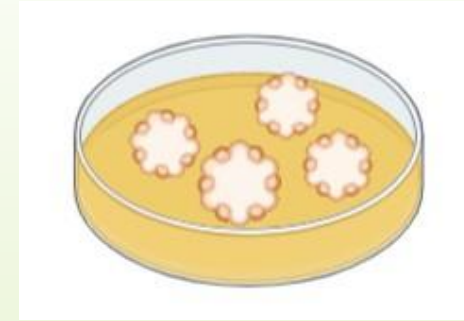
- Human glioma cell lines (U373, U87)
- Neurons differentiated from neuroblastoma or iPSC

EXPERIMENTAL MODEL



Alzheimer's disease model

«Alzheimer-like» cerebral organoids



Gliomas model

Tumoroids of gliomas



Alzheimer's disease and Gliomas models

PATHWAYS

- **Antioxidant response:**

- ROS;
- Calcium;
- GSH/GSSG ratio;
- Analyses of Nrf2/ARE gene pathway

- **Iron homeostasis pathway:**

- Iron levels;
- Lipid peroxidation;
- Key proteins (GPX4, System Xc);
- Iron carriers (TfR, FPN);
- Cell survival

METHODS

- Fluorescent probes: DCFH-DA; Fluo-4 Direct Calcium assay kit; BODYPY 581/591 C11; BioTracker FerroOrange live cell dye
- Colorimetric kit: Glutathione GSH/GSSG assay kit
- Western blot and immunofluorescence
- RT qPCR
- MTT assay

Alzheimer's disease and Gliomas models

PATHWAYS

- **Organelles function:**

- Measure ATP levels and mDNA copy number;
- Analyses of endolysosomal pH;
- CD44/HA
- Expression and localization of keys proteins (GM130, TRPML1)

- **Inter-organelles communication:**

- Evaluation of the membrane contact sites

METHODS

- Seahorse xf hs mini analyzer
- RT qPCR
- pH-sensitive fluorescent probes
- Western blot and immunofluorescence
- Confocal analyses

- Transfection with expression vector and confocal analyses

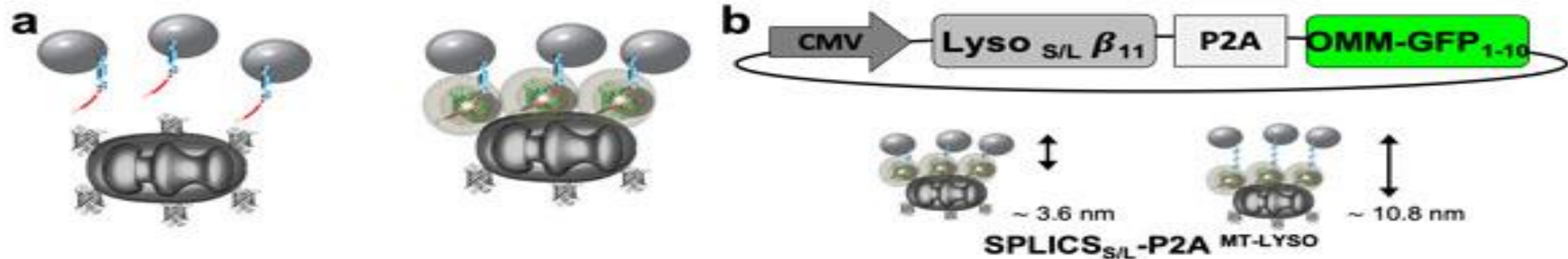
Alzheimer's disease and Gliomas models

PATHWAYS

- **Inter-organelles communication:**
 - Evaluation of the membrane contact sites

METHODS

- Transfection with expression vector and confocal analyses



Alzheimer's disease and Gliomas models

PATHWAYS

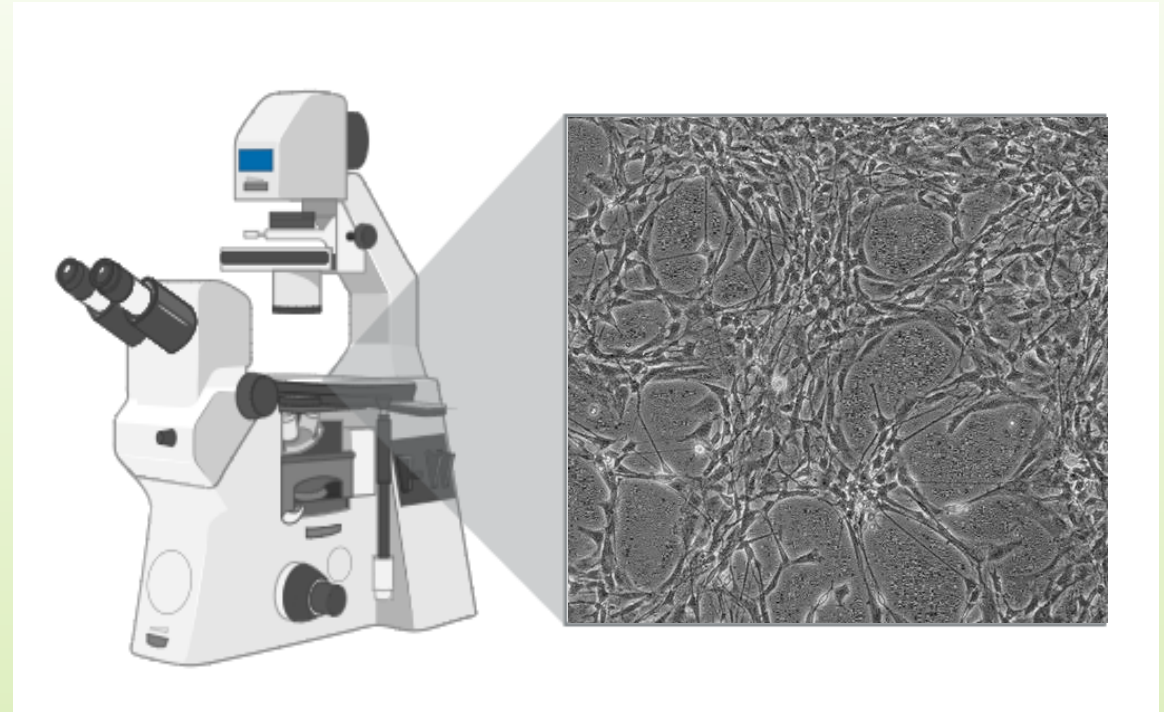
- **Crosstalk between astrocytes and neurons:**
 - Evaluation of released soluble factors and extracellular vesicles;
 - Analysis of the viability of neurons exposed to these factors

METHODS

- ELISA assay
- Western blot
- Microarray assay
- MTT assay

EXPECTED RESULTS

The dissection of the pathways activated in astrocytes during pathological conditions, could lead to the identification of new therapeutic targets



THANK YOU FOR YOUR ATTENTION!