In vitro stable culture system of cerebellar granular cell precursors (GCPs), stable method for in vitro culturing of the mentioned cells and its applications.

KEYWORDS

- ❑ STABLE CULTURE
- ❑ GRANULE CELL PRECURSORS (GCPS)
- ❑ CEREBELLUM
- ❑ 3D NEURONAL CULTURE
- ❑ NEUROSPHERES
- ❑ SHh PATHWAY
- ❑ MEDULLOBLASTOMA

AREA

- ❑ CHEMISTRY & BIOTECHNOLOGY

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Patent Type
Patent for invention.

Co-Ownership
Sapienza Università di Roma 80%, Fondazione Istituto Italiano di Tecnologia 20%.

Inventors
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Industrial & Commercial Reference
Industries that produce cell models in vitro.

Time to Market
TRL 4= technology validated in lab.

Availability
Cession, Licensing, Research, Development, Experimentation, Collaboration and Spin-off.

GCPs culture methods	pros	cons
Transient culture of primary cerebellar granule cell precursors	Physiologic system	Short half-life in vitro, limited material
Medulloblastoma cell lines	Stable culture	No Physiologic system
Cell lines engineered with constructs expressing components of the Shh pathway	Stable culture	No neuronal context, usually fibroblasts
Neurospheres cultures from cerebellar explants (with bFGF)	Stable culture	In culture bFGF inhibits the Shh pathway
Neurospheres cultures from cerebellar explants (with SAG)	Physiologic system	2 weeks of half-life in vitro
Neurospheres cultures from cerebellar explants (with our new mix of growth factors)	**Physiologic system and Stable culture. No time limitation**	

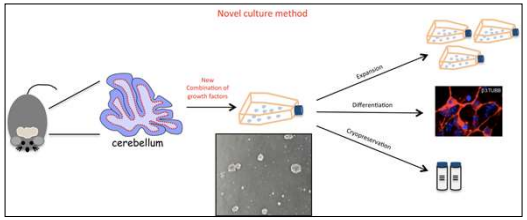

Fig. 1 Schematization of our new culture method: GCPs explanted from mice are grown in vitro as neurospheres and in this form expanded, differentiated and cryopreserved.

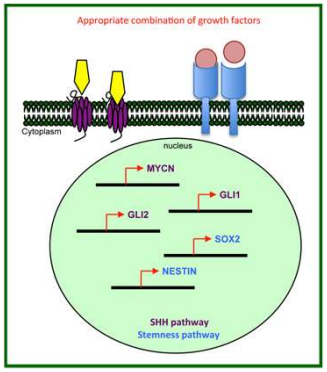

Fig. 2 The new combination of growth factors applied in our cultures ensures that GCPs express both Sonic Hedgehog and stemness pathway markers, in vitro.

Abstract
The present invention refers to a stable in vitro culture system of cerebellar granule cells precursors (GCPs). The culture system maintains a high expression of the Sonic Hedgehog pathway in the GCPs and an unlimited proliferative capability guaranteed by high levels of the Sox2 and Nestin stem factors. For these reasons, the aforementioned culture system can be a valid model in vitro for the study of the pathophysiology of the cerebellar granules, for the study of cerebellar diseases consequent to damage or neurodegeneration and potentially for their treatment through gene therapy approaches.

Pubblicazioni
SMO-M2 mutation does not support cell-autonomous Hedgehog activity in cerebellar granule cell precursors. M. Petroni and M. Sahùn Roncero, et al. Scientific reports. https://doi.org/10.1038/s41598-019-56057-y

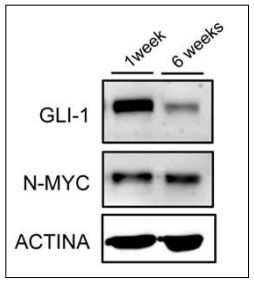

Fig. 3 GCPs cultured according to our protocol maintain the expression of the Sonic Hedgehog pathway over time. Gli1 and N-Myc are known markers of this pathway and actin is used as a loading control.

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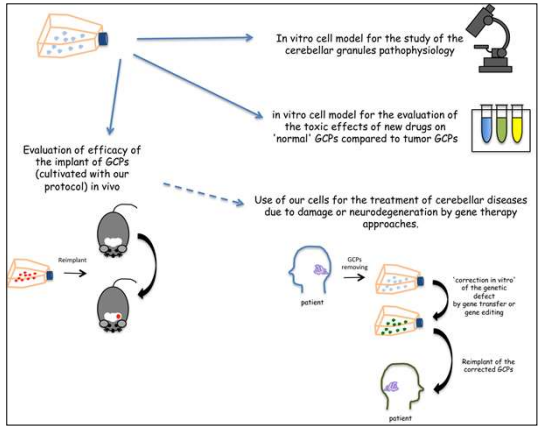
Technical Description

After surgical resection at the 7th day of postnatal life, the mouse cerebellum is mechanically shredded in a buffer containing HBSS, Glucose and antibiotics. The individual cells obtained are seeding in a medium composed of DMEM-F12, Glucose, B27 W/O VitA, insulin, NALC, heparin, antibiotics and growth factors.
In this medium, the cells can be expanded and maintained for an unlimited time. Periodic dissociation of the spheres and propagation by dilution is needed to prevent excessive growth leading in cell death caused by the lack of nutrients and hypoxia.



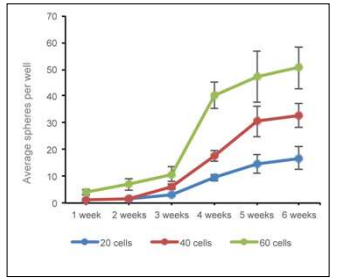
Technologies & Advantages

The proper functionality of the cerebellum dependents on the expansion, migration and differentiation of GCPs. In fact, defects in the molecular pathways that regulate these processes are responsible for different pathological conditions (https://medlineplus.gov/cerebellardisorders.html), including medulloblastoma.
The lack of appropriate in vitro models that fully preserve the physiological characteristics of the GCPs still remaining 'permissive' to the expression of their main pathways (i.e. Shh pathway), has made the studies published so far incomplete and questionable. Indeed, GCPs grown according to already known culture models quickly stop proliferating, thus requiring generation of ex-novo cultures for each experiment; alternatively, they may be of tumor origin and therefore unsuitable to correctly represent GCPs physiology.
The present invention describes a new protocol which allows to obtain GCP cultures in a state of unlimited proliferation and with a gene / biochemical expression profile comparable to that of fresh GCPs cultures, even after freeze&thaw procedures.

Applications

1. Generation of in vitro models for the study of the pathophysiology of cerebellar granules;
2. Evaluation in vitro of the toxic effects of new drugs on 'normal' GCPs compared to tumor GCPs before in vivo studies;
3. Implementation of the invention towards its use for the treatment of cerebellar diseases due to damage or neurodegeneration, by gene therapy approaches. GCPs cultures prepared according to our method could be expanded in vitro and re-implanted in the cerebellum. In principle, GCPs from sick individuals could be 'corrected in vitro' by gene transfer or gene editing of the genetic defect, expanded in vitro according to our method, and consequent replanted in the original cerebellum.

Fig. 4
GCPs cultured according to our protocol maintain clonogenicity (marker of proliferation and cell health) over time.



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