Glia isolated from adult gut generates progenitors of the enteric nervous system: an alternative source of replacement cells for regenerative strategies

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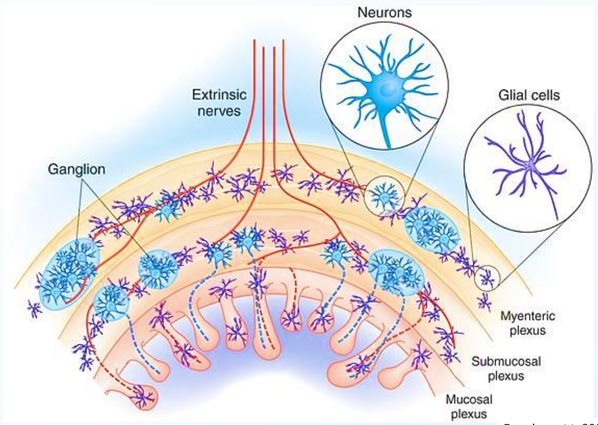
Background: Nerve tissue is not only present in the brain but also in the periphery. The gastrointestinal tract contains a network of nerves called the enteric nervous system (ENS, the "second brain"). The ENS comprises ganglia containing adult neurons and glial cells, and neural precursor cells.

Glia from fetal and postnatal gut cultures is able to generate ENS progenitors. Glia in the adult ENS maintains a limited neurogenic potential, which is activated in culture and in response to injury *in vivo*.

AIM: We evaluated whether the glia-generating-progenitor potential does exist in the adult gut and in physiological conditions in vitro.

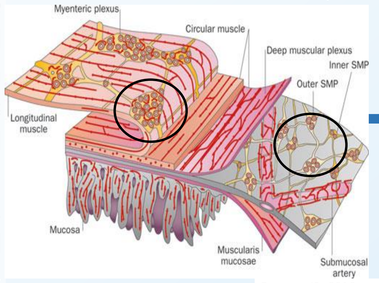


Rescigno M, 2008

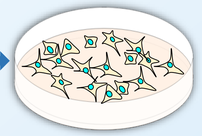
Methods: Primary glial cells were isolated from rat and human ileum and cultured with glia medium (DMEM F12, fetal calf serum 10%) until confluence (day 14). Glial cells were then trypsinised and cultured in non-adherent flasks with proliferation medium [DMEM F12, containing growth factors (basal-fibroblast growth factor-bFGF and epidermal growth factor-EGF)].

Cultures were characterized by immunostaining, using specific markers for glial cells (S100B, glial fibrillary acidic protein-GFAP, proteolipid protein-PLP-1), neurons (HuCD and neurofilament-NF-200) and neural progenitors (nestin and Tuj1).

Intestinal Layers

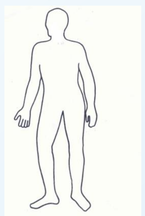


Furness JB, 2012

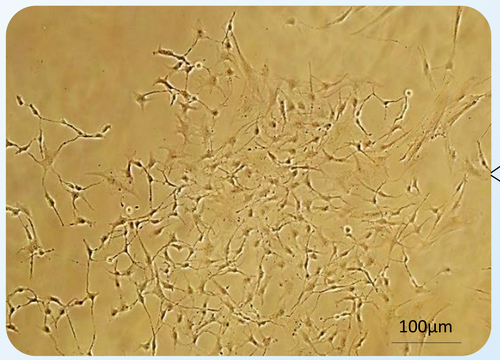

Enteric Glia culture

RAT

HUMAN

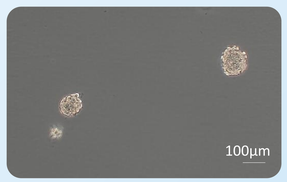


Amplification of primary adult ENTERIC GLIA

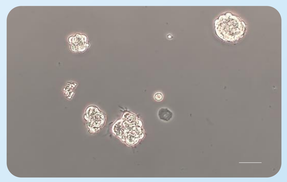


bright field

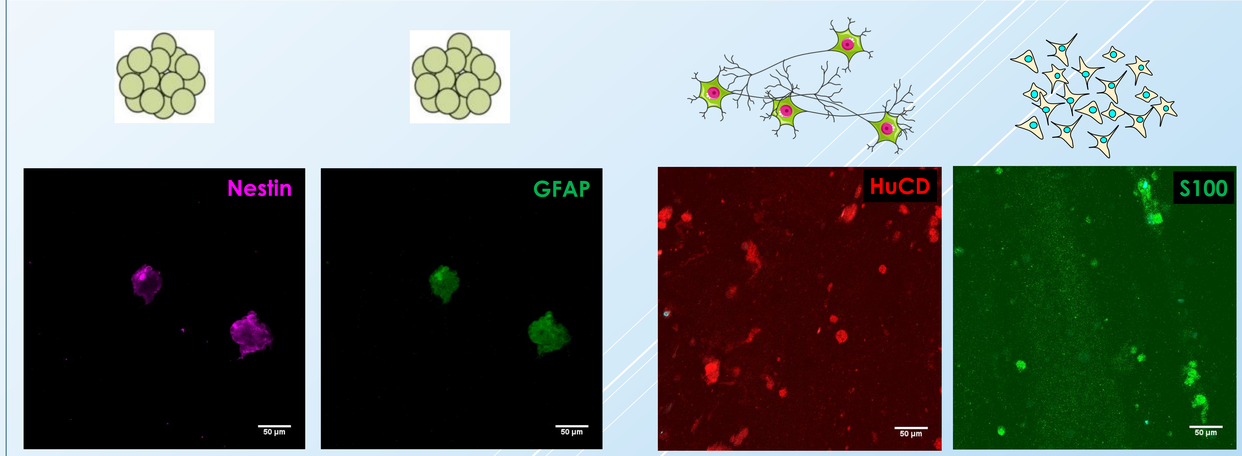
Growth factors



Primary neurospheres


Secondary neurospheres

Results: Cultures of dissociated adult glia gave rise to neurosphere-like bodies when cultured with growth factors. In these conditions, glia cultures started to pile-up and form spherical structures which detached and floated in the medium. Primary glia-generated neurosphere-like bodies generated secondary and tertiary cultures. Immunostaining revealed that neurospheres contained progenitor cells. When glia-generated neurosphere-like bodies were cultured with specific media to induce differentiation *in vitro*, they gave rise to cultures containing neurons and glial cells.



Conclusion: Our findings establish the feasibility of expanding ENS progenitors starting from adult primary glia cultures from rat and human. Glia in the adult ENS can be looked at as a promising source of replacement cells for regenerative strategies.