Human neuro-mesodermal assembloids recapitulate aspects of peripheral nervous system development in vitro

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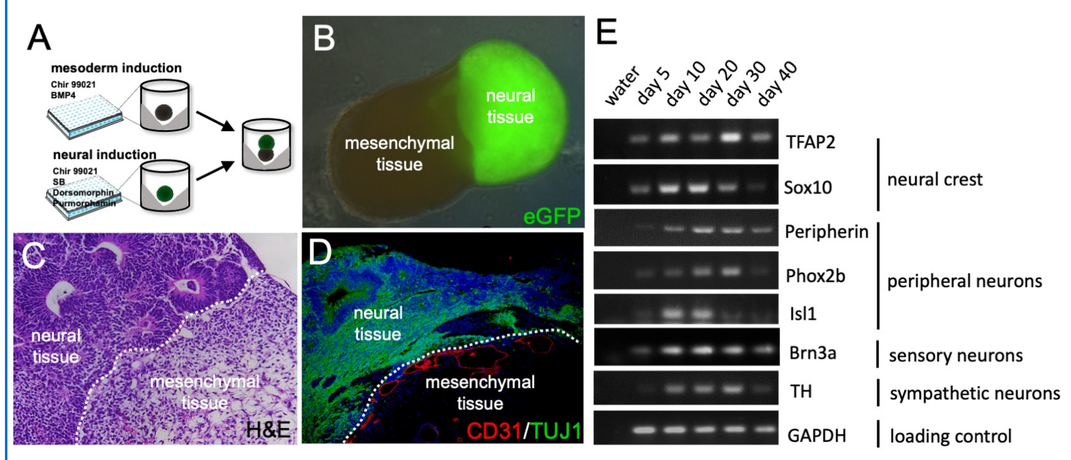
Introduction

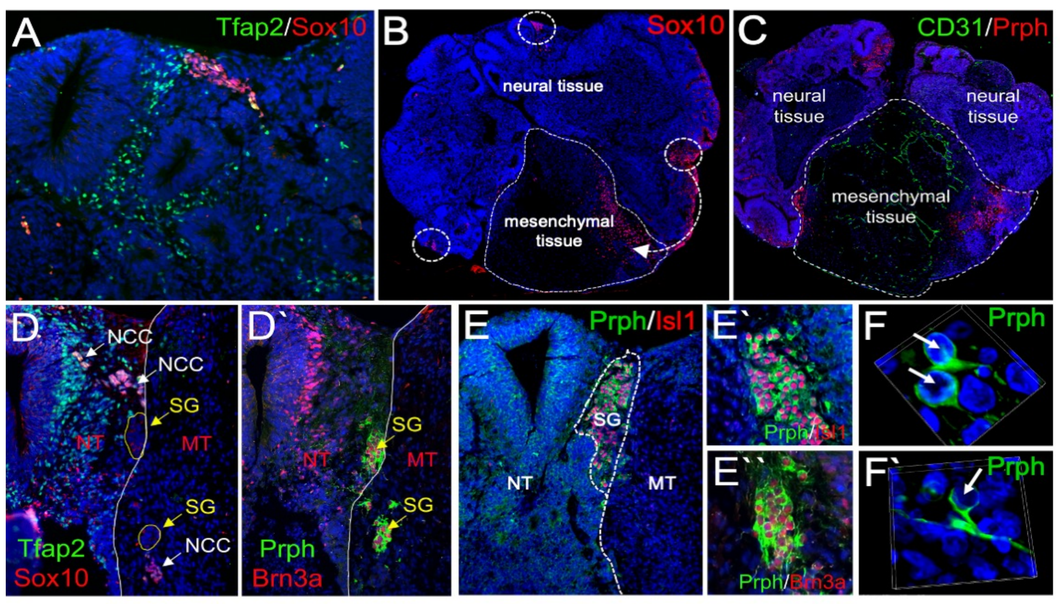
Within the last decade, numerous human stem cell-based 3D cell culture models were explored, mimicking the development of different organs and tissues. These tissue models are termed organoids. To increase tissue complexity and allow multi-lineage interaction, different organoids can be combined forming assembloids.
The aim of the presented work is the establishment of a novel neuro-mesodermal assembloid model which recapitulates aspects of peripheral nervous system (PNS) development. To reach this goal, assembloids are produced by co-culturing neural and mesodermal spheroids which are separately generated from human induced pluripotent stem cells.

Neuro-mesodermal assembloid generation



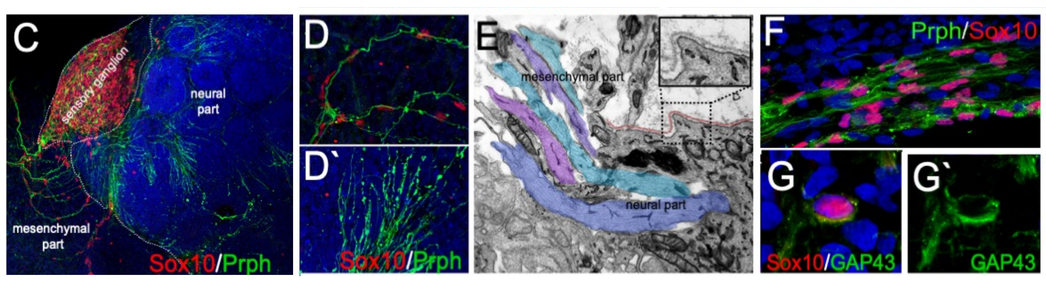
A: Experimental workflow. **B:** Depiction of a typical neuro-mesodermal assembloid. **C:** H&E-stained histological section of a neuro-mesodermal assembloid at day 14. **D:** Immunofluorescence analysis of neuro-mesodermal assembloid. Specific antibodies targeted against the endothelial marker-protein PECAM (CD31) and the neuron-specific marker-protein b-III tubulin (TUJ1) were used. **E:** Semiquantitative RT-PCR analyses detecting neural crest (TFAP2, Sox10), peripheral neuron (Peripherin, Phox2b, Isl1) and sensory (Brn3a) as well as sympathetic neuron (TH) markers.

NCC migration and sensory ganglion formation



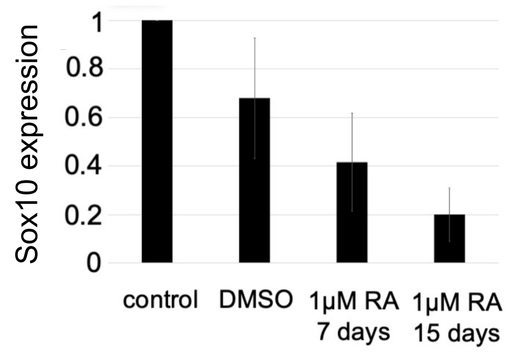
Immunofluorescence analysis on neuro-mesodermal assembloids **A:** Detection of TFAP2 and Sox10 expression. **B:** Detection of Sox10 expression shows neural crest migration. **C:** Peripherin⁺ cells at the neuro-mesodermal interface indicating PNS-neurons . CD31⁺ expression is visible in the mesodermal part. **D-D`:** TFAP2⁺/Sox10⁺ neural crest cells (NCC) migrate from the neural (NT) into the mesodermal (MT) part of the assembloid. Peripherin⁺/Brn3a⁺ sensory ganglia (SG) form near the neuro-mesodermal interface. **E-E``:** Cells within sensory ganglia co-express Peripherin, Brn3a and Isl1. **F-F`:** Sensory neurons display pseudo-unipolar morphology.

Schwann cells and peripheral nerve formation



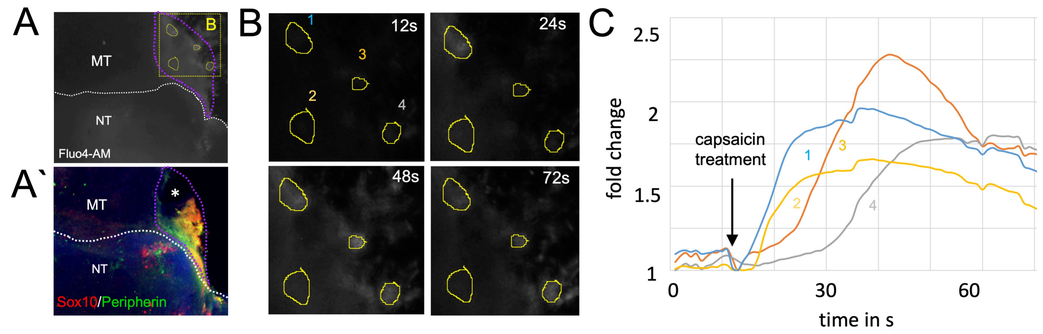
C: 3D reconstruction of immunofluorescence pictures taken from tissue cleared assembloid. A Sox10/Peripherin⁺ ganglion is depicted sending axonal projections to the neural and the mesodermal part of the assembloid. **D:** Axons projecting to the mesodermal part are accompanied by Sox10⁺ Schwann cell-like cells (D). Axons projecting to the neural tissue are devoid of Sox10⁺ cells (D´). **E:** Transmission electron microscopic (TEM) analysis reveals axons (blue, purple and green color) penetrating the basement membrane (brown color) at the neuro-mesodermal interface marking the basal side of the neuroepithelium. **F-G:** Peripheral nerve-like axon bundles are accompanied by Sox10/GAP43⁺ Schwann cell-like cells.

Impact of retinoic acid on NCC migration



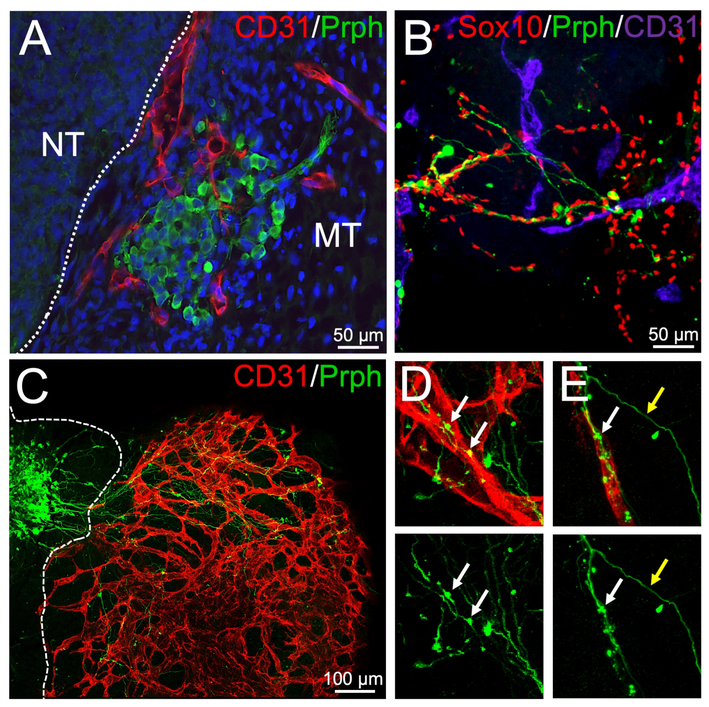
Treatment of assembloids with 1 µM retinoic acid (RA) reduces Sox10 expression in a time dependent manner. The solvent DMSO also showed a mild effect on Sox10 expression.

Sensory neurons are responsive to Capsaicin



A: Assembloids were loaded with the calcium indicator Fluo4-AM and treated with capsaicin. Elevated calcium levels in response to capsaicin treatment were observed in a distinct region of the organoid (purple dotted line). **A`** After calcium measurement, assembloids were stained for Sox10 and Peripherin, and tissue cleared. 3D reconstruction of confocal immunofluorescence images confirmed the location of a peripheral ganglion in the capsaicin responsive region. Tissue was partially destroyed during sample preparation (white asterisk). **B:** Higher magnification of the image depicted in A (yellow dotted square). Pictures show Fluo4-AM fluorescence at different time points (12s, 24s, 48s, 72s). **C:** Quantification of Fluo4-AM fluorescence intensity in 4 selected regions of interest (ROI) (yellow circles in image A and B)

Interaction of blood vessels and peripheral neurons



A: Peripherin⁺ ganglia are enwrapped by a CD31⁺ endothelial network. **B:** Migrating neural crest cells and peripheral neurons in close contact with vascular structures. **C:** Tissue cleared assembloid. A sensory ganglion (green) and a well-organized vascular plexus (red) are observed. **D-E:** Peripheral nerve fibers align with blood vessels and form bouton en passant-like structures (white arrow). These structures are not observed in axons without vessel contact (white arrow).

Conclusion

The presented assembloid model could help to uncover mechanisms of NCC delamination, migration and PNS development in the human tissue context. Moreover, the model could be used for toxicity screenings or drug testing. The co-development of mesodermal and neuroectodermal tissues and of a well-organized vascular plexus along with a peripheral nervous system allows to investigate the crosstalk between neuroectoderm and mesoderm and between peripheral neurons/neuroblasts and endothelial cells. Such interactions influence NCC delamination and migration, sensory neuron differentiation and rearrangement of the primitive vascular plexus in the embryo.

Supplemental movies and material

Organoid with Blood vessels (CD31 – red) and sensory nerves (Prph – green).

Organoid with Blood vessels (CD31 – red), sensory nerves and the formation of ganglion-like structures (Prph – green), (DAPI).

Calcium imaging (Fluo4 AM) after Capsaicin treatment

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