PHENOTYPIC SCREENING OF HUMAN STEM CELL-DERIVED OLIGODENDROCYTE PROGENITORS FOR THE DISCOVERY OF REGENERATIVE REMYELINATING DRUGS

PROGENITORTHERAPEUTICS regenerative drug discovery

Milena Mennecozzi[1], Katrina McCarten[1], Marina Tarunina[1], Yen Choo[1,2]

[1]Plasticell Ltd, [2]Progenitor Therapeutics Ltd, Stevenage BioScience Catalyst, Stevenage, UK

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1) Abstract

The demyelinating disease multiple sclerosis (MS) affects around 2.5 million people worldwide. In around 70% of the MS patients, oligodendrocyte precursor cells (OPCs) are recruited to damaged lesions but fail to differentiate into myelin producing oligodendrocytes. Identifying drugs that enhance the differentiation and maturation of the resident OPC population to myelin producing oligodendrocytes could have important therapeutic implications.

We have identified a number of efficient *in vitro* protocols capable of generating PDGFRa$^+$ OPCs from human neural stem cells in monolayer. These protocols were identified via the use of CombiCult®, Plasticell's bead-based combinatorial cell culture system, which allowed us to screen 6,912 combinations of unique cell culture media. CombiCult® derived OPCs were characterised for their differentiation potential, protein markers expression, mRNA expression and hence suitability for screening. The OPCs will then be used in a phenotypic screen to identify compounds which generate myelin positive oligodendrocytes.

2) Neural stem cell characterisation

➢ Human neural stem cells were seeded in 96 well plates and stained with Hoechst (blue) plus stem cells or OPC markers (green).

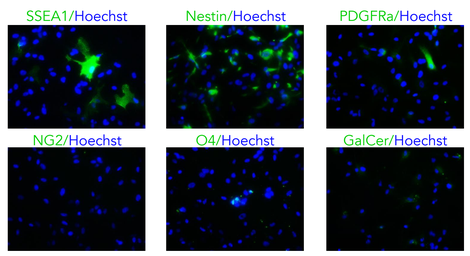


Fig 1. Neural stem cells express SSEA1 and Nestin and do not express OPC markers (NG2, O4, and GalCer) as analysed by immunocytochemistry staining.

3) Neural stem cells differentiate into 3 lineages

➢ Human neural stem cells were differentiated into neurons, oligodendrocytes, and astrocytes using published protocols.

➢ Cells were immunostained with Hoechst (blue) and bIII-Tubulin, GalCer, and GFAP (green).

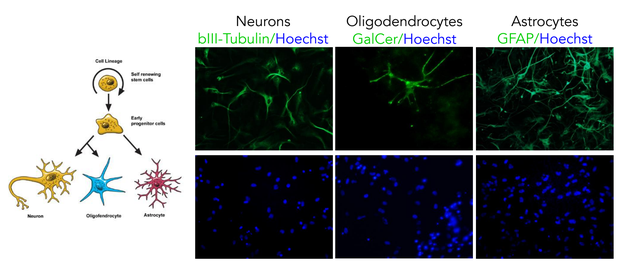
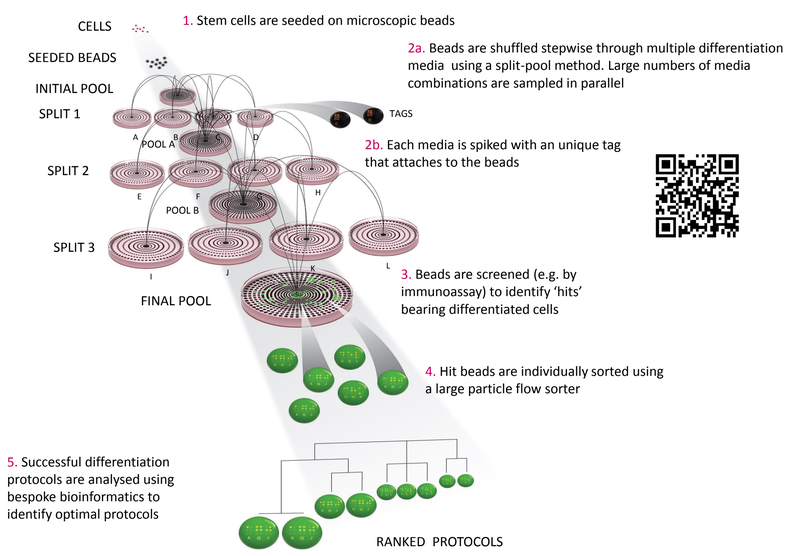


Fig 2. Human neural stem cells can differentiate into bIII-Tubulin$^+$ neurons, GalCer$^+$ oligodendrocytes, and GFAP$^+$ astrocytes.

4) Combinatorial cell culture (CombiCult®) platform

➢ Combinatorial cell culture (CombiCult®) is a bead-based, combinatorial technology specifically developed for discovery of novel stem cell differentiation protocols.

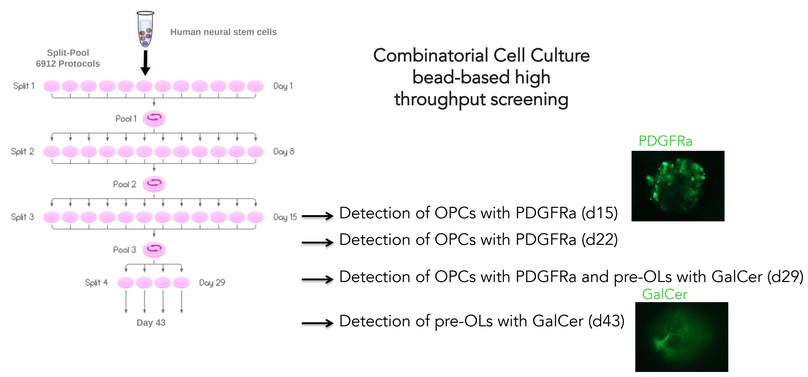


5) Identification of protocols that differentiate human neural stem cells into PDGFRa$^+$ and GalCer$^+$ cells

➢ CombiCult® was used to screen 6,912 protocols to identify media capable of generating OPCs and pre-OLs from human neural stem cells.

➢ Human neural stem cells were grown on microcarriers and shuffled randomly through 40 culture conditions with concomitant labelling of the beads using nanomaterial tags.

➢ CombiCult® screen resulted in the identification of 24 media combinations capable of making PDGFRa$^+$ and/or GalCer$^+$ cells.

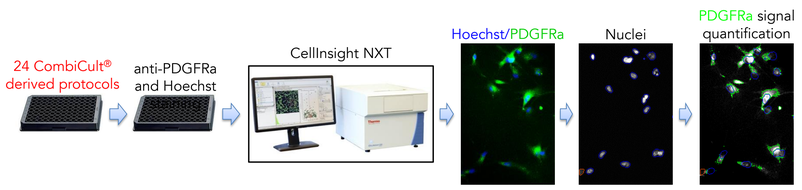


6) Validation of selected protocols using monolayer human neural stem cell culture

➢ Human neural stem cells were seeded in 96 well plates and exposed to 24 CombiCult® derived protocols.

➢ Cells were fixed and stained by immunocytochemistry with markers such as PDGFRa and O4.

➢ Fluorescent signal intensity was quantified using automated microscope (CellInsight NXT).



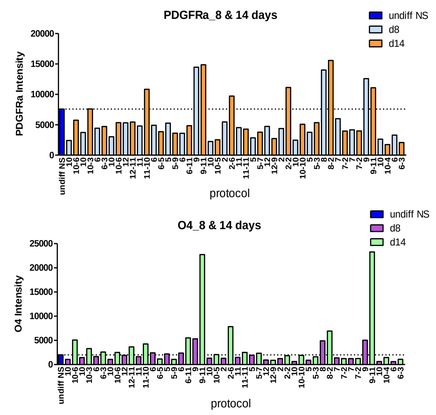


Fig 3. At day 14, human neural stem cells exposed to protocols: 9-11, 2-6, and 8-2 show an enrichment in PDGFRa and O4 protein expression as analysed by immunocytochemistry and high content imaging.

7) 8-2 OPC characterisation

➢ Human neural stem cells were seeded in 6 well plates and exposed to CombiCult® derived media combination 8-2 for 14 days.

➢ Undifferentiated neural stem cells and OPCs were stained with PDGFRa.

➢ RNA was extracted and the expression of OPC and OL markers was evaluated by qPCR.

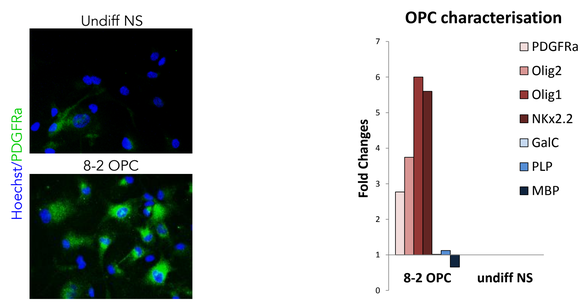
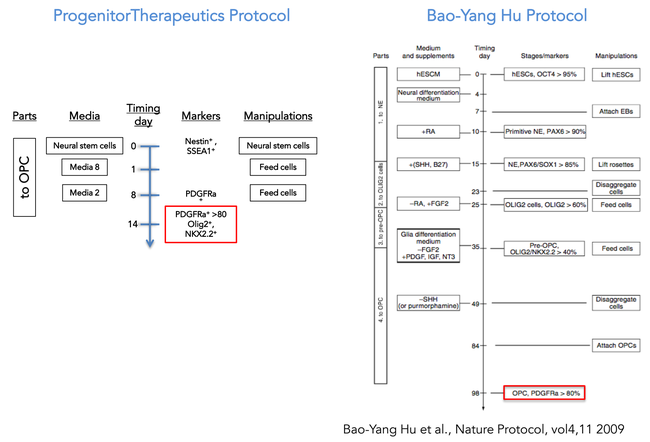


Fig 4. Cells generated by exposing human neural stem cells to media combination 8-2 express OPC markers such as PDGFRa, Olig2, Olig1, and NKx2.2. Pre-OL and OL markers (GalC, PLP, and MBP) are not expressed.

8) Rapid and efficient generation of OPCs



Bao-Yang Hu et al., Nature Protocol, vol4,11 2009

Fig 5. OPCs expressing PDGFRa, Olig2, and NKx2.2 are obtained in 14 days exposing human neural stem cells to CombiCult® derived media combination 8-2.

9) 8-2 OPCs differentiate into MBP$^+$ cells

➢ 8-2 derived OPCs were exposed to T3 + miconazole for 20 days.

➢ MBP expression was assayed by immunostaining.

➢ qPCR was performed to evaluate the expression of OPC and OL markers.

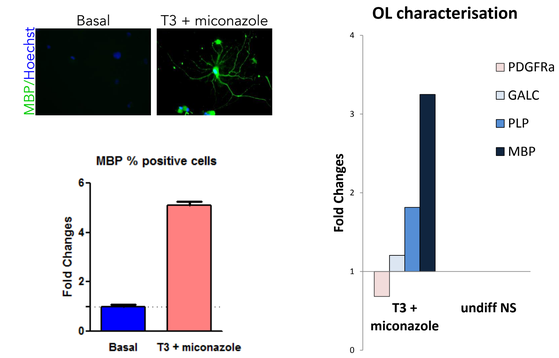


Fig 6. 8-2 derived OPCs exposed to T3 + miconazole express MBP protein and assume a typical OL morphology. qPCR analysis confirms MPB expression and shows an increase in PLP signal in cells generated using T3 + miconazole.

10) Phenotypic screening scheme

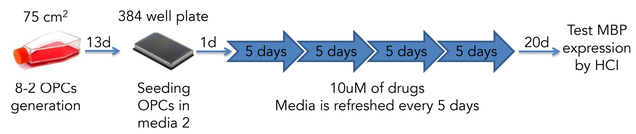
8-2 CombiCult® derived OPCs were used in a small molecule phenotypic screen aimed to identify new regenerative drugs for multiple sclerosis:

➢ Human neural stem cells, seeded in 75 cm^2 flask, were exposed to media combination 8-2 for 13 days to derive OPCs.

➢ At day 13 8-2 derived OPCs were collected and seeded in 384 well plate in media 2 and, after one day, exposed to 10uM small molecules libraries for 20 days (drugs were refreshed every 5 days).

➢ At day 20, the cells were fixed, stained, and imaged by HCI.

➢ Hits were identified and are going to be validated in dose response.



11) Conclusions

➢ We have described the discovery of novel serum-free protocols for the generation of OPCs from human neural stem cells using CombiCult® technology.

➢ CombiCult® derived protocols were validated in cells grown on monolayer.

➢ 8-2 CombiCult® derived media combination was identified as the best protocol to generate Olig2$^+$/PDGFRa$^+$ OPCs in 14 days.

➢ OPCs generated using 8-2 media combination can differentiate into MBP$^+$ cells in 20 days using T3 + miconazole.

➢ Phenotypic screening to identify new myelinating regenerative drugs has been established in 384 well plates.

Acknowledgement

We thank GSK for compound libraries. The human biological samples used in this study were sourced ethically and their research use was in accord with the terms of the informed consents. We would like to thank Dr David Lee (Scinovo, GSK) and Dr Martin Ruediger (GSK) for providing support and services.

www.progenitortherapeutics.com Plasticell Ltd / Progenitor Therapeutics Ltd, Stevenage BioScience Catalyst, GSK campus, Gunnels Wood Road, Stevenage SG1 2FX, United Kingdom [Correspondence: milena@plasticell.co.uk] www.plasticell.co.uk