Our panel of experts highlight the most important research articles across the spectrum of topics relevant to the field of regenerative medicine

Synergistic effect of PDGF & FGF2 on dermal papilla cells *in vitro* – a hope for cell therapy of human hair follicle regeneration

Evaluation of: Kiso M, Hamazaki TS, Itoh M *et al.* Synergistic effect of PDGF and FGF2 for cell proliferation and hair inductive activity in murine vibrissal dermal papilla *in vitro*. *J. Dermatol. Sci.* 79,110–118 (2015).

Follicular cell implantation emerges as a promising cell therapy for hair loss, in which dermal papilla cells are dissected from scalp tissue biopsies and expanded *in vitro*. Expanded dermal papilla cells are then transplanted either with follicular keratinocytes or alone onto a patient's head to regenerate new hair follicles [1]. However, it has been very challenging to expand dermal papilla cells while maintaining their inductive potential *in vitro* and one of the solutions was to utilize conditioned medium from keratinocytes [2]. In this article, Kiso and colleagues found that two cytokines, PDGF and FGF2, can work together to maintain the hair inductive potential of mouse dermal papilla cells *in vitro*, as demonstrated by two hair inductive assays, the subcutaneous patch assay and chamber assay. This result raises a great hope that inductive human dermal papilla cells could be expanded simply in the presence of these two growth factors to generate enough cells for a follicular cell implantation therapy for hair loss in clinic. To take this claim, it is still necessary to confirm the harmonic function of PDGF and FGF2 on human dermal papilla cells, although it has been demonstrated that human dermal papilla cells induced hairs when they were maintained in the keratinocyte conditioned medium [3].

Interestingly enough, another article concluding that PDGF signaling in the dermis and in dermal condensates is indispensable for hair follicle induction and formation was recently published by Rezza *et al.* of Mount Sinai of New York [4]. They found that PDGF signaling was not required for hair follicle morphogenesis because hair follicle induction and development were completely normal despite conditional ablation of both PDGF receptors in early dermal condensates and the ventral dermis. One explanation for these 'conflicting' results would be that both studies primarily emphasized that growth factors work in concert to exert biological functions. Kiso *et al.* demonstrated that dermal papilla cells treated with FGF2 alone did not induce hair in subcutaneous injection assays or sparse hairs in chamber assays; it was only the combination of both PDGF and FGF2 that induced hairs in both assays. In this study, it was not shown if dermal papilla cells treated with PDGF alone would induce hairs. It would be very interesting to see that PDGF-treated dermal papilla cells do not induce hairs, so that both publications would support each other. This would result in an important therapeutic finding for hair follicle regeneration and in understanding the mechanism of hair follicle development and morphogenesis.

Lesion-induced accumulation of platelets promotes survival of adult neural stem/progenitor cells

Evaluation of: Kazanis I, Feichtner M, Lange S *et al.* Lesion-induced accumulation of platelets promotes survival of adult neural stem/progenitor cells. *Exp. Neurol.* 269, 75–89 (2015).

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Harnessing the endogenous capacity of regeneration after CNS injury has been studied in great detail; however, very few answers have arisen. Neural stem cells and progenitor cells support the turnover of cells in addition to little regeneration. Recently, platelets have been implicated in promoting repair after injury. Platelets express many surface molecules which are able to interact with glia and neurons of the CNS after injury. The researchers in this current paper utilized platelet lysate which contains a large number of chemokines, cytokines and growth factors as well as RNA molecules and micro particles. The researches utilized the lysolecithin model of demyelination in the corpus callosum of the brain and investigated the role of platelets in the sequestration of cells in the subependymal zone (SEZ).

Kazanis and colleagues assessed the lesion area and the laminin positive vascularity which coexists within the lesion. 1 week and 15 days post-lesion, the density of laminin expressing vessels increased in the ipsilateral ventricle. Early post lesion, platelets were found to accumulate in the area of the lesion attaching to vessel walls and migrating out of the vasculature. This phenomenon was not detected on the contralateral side. Accumulations of platelets were also discovered in the lateral ventricle walls and SEZ in the ipsilateral and contralateral walls in close proximity to proliferating stem cells (Ki67⁺, nestin⁺). Platelets are activated early in lesioning and become in close proximity to stem cells and the SEZ vasculature. The molecules involved after the platelets are released come into question. Therefore, they used platelet lysate to determine if addition into *in vitro* cultures of neural stem cells and they do indeed affect morphology or expansion. With increased dosing of platelet lysate, the neural stem cells attach to the plastic surface and change morphology. The platelet lysate regardless of concentration did not affect the cell differentiation. The neural stem cells exposure to platelet lysate did not express terminal differentiation markers. The platelet lysate used in these studies did not affect the percentage of oligodendrocyte, astrocytic or neuronal markers. More *in vitro* studies were carried out and they determined the proliferation rate importantly remained unchanged in cultures with the platelet lysate. Further, apoptosis seemed to be much lower in the cultures grown in the medium containing platelet lysate. The lesion mouse tissue was also determined to have less apoptosis in the region of pooled nestin positive cells in relation to platelets in the SEZ. However, this may be a coincidence. The relation of the cells needs to be monitored more closely.

The researchers in this study indicated an importance of platelets in injury and their role may be closely related to the stem cells in close proximity to a CNS

injury. However, most of their work was done in cell culture and may not translate well into the animal model. The researcher's next steps should be to implant platelet lysate into the animal model and determine whether this enhances or is detrimental to repair.

Cell lysis contributes to the potency of allogeneic MSCs

Evaluation of: Aizman I, Vinodkumar D, McGrogan M, Bates D. Cell injury-induced release of fibroblast growth factor 2: relevance to intracerebral mesenchymal stromal cell transplantations. *Stem Cells Dev.* 24(14), 1623–1634 (2015).

Mesenchymal stromal cells have been suggested as a therapy for myriad degenerative disorders, including those afflicting the central nervous system. Historically, the therapeutic effects of MSCs have been attributed to the release of soluble factors following engraftment at the site of injury. This mechanism of action was implicated even with the transplant of allogeneic MSCs, which were long thought to be immunoprivileged. More recently, it has become clear that engraftment rates of allo-MSCs are low, which has been ascribed mostly to the fact that these cells are rejected by innate and adaptive immunity. Regardless of the mechanism of graft loss, the therapeutic effects of the implanted cells can remain, long after the cells have disappeared. In the current work, Aizman *et al.* demonstrate that the clinically-observed benefits of transplanted allogeneic MSCs (SB623) may be the result of the release of soluble factors, in particular FGF2, upon cell death, and that these soluble factors can work synergistically with a small population of living engrafted cells to carry out their therapeutic effect.

The authors first demonstrate that the levels of FGF2 released on freeze/thaw lysis of SB623 cells is 230-fold higher than it is in conditioned medium (MSC-CM), which was not true of VEGF and MCP1, which were largely concentrated extracellularly. An important bridge to physiology was demonstrated in a cytotoxic PBMC experiment, where similar levels of FGF2 were released after lysis in MSC/allo-PBMC cocultures. BrdU labeling of HUVECs cultured in the presence of SB623-CM and SB623 freeze/thaw extracts demonstrated that cells proliferated most in the extract; addition of an FGF2 neutralizing antibody reversed this effect, demonstrating that FGF2 was a primary factor in the observed proliferation, and by extension, that release of FGF2 upon cell lysis represents a strong potentiator of angiogenesis *in vivo*. Proliferation of neural progenitors was also observed to be higher with extracts than with CM. Neurogenesis, as measured by rat Nestin expression, was enhanced

by both the CM and extract samples, whereas astrogenesis was only potentiated by a combination of live cells and extract. It is known that that FGF2 is active predominantly as a complex with heparin or heparin sulfate proteoglycans [5], so it would have been interesting to see how the potency of the extract would have been effected had the SB623 culture been grown in the presence of these polysaccharides or a hydrolyzed extracellular matrix.

This work is an important step toward understanding the mechanisms of action of cellular therapies, in particular allogenic cells, in light of a new understanding of their persistence and engraftment following administration. It is plausible that other allogeneic cell therapies rely of cells as a depot of growth factors for their mechanism of action. For example Apligraf,

an allogeneic living cellular construct comprising fibroblasts and keratinocytes, does not engraft, but its wound healing effect persists long after the cellular components of the construct are no longer present in the wound [6].

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