Stem Cell and Transdifferentiation Study Literatures

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Abstract: Transdifferentiation is a process where one mature somatic cell transforms into another mature somatic cell without undergoing an intermediate pluripotent state or progenitor cell type. It is a type of metaplasia, which includes all cell fate switches, including the interconversion of stem cells. Current uses of transdifferentiation include disease modeling and drug discovery and in the future may include gene therapy and regenerative medicine. Somatic cells are first transfected with pluripotent reprogramming factors temporarily before being transfected with the desired inhibitory or activating factors. Stem cells are undifferentiated biological cells that can differentiate into specialized cells and can divide to produce more stem cells. They are found in multicellular organisms. In adult organisms, stem cells and progenitor cells act as a repair system for the body, replenishing adult tissues.
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Key words: transdifferentiation; stem cell; eternal; life; organism

1. Introduction

Transdifferentiation is a process where one mature somatic cell transforms into another mature somatic cell without undergoing an intermediate pluripotent state or progenitor cell type. It is a type of metaplasia, which includes all cell fate switches, including the interconversion of stem cells. Current uses of transdifferentiation include disease modeling and drug discovery and in the future may include gene therapy and regenerative medicine. Somatic cells are first transfected with pluripotent reprogramming factors temporarily (Oct4, Sox2, Nanog, etc.) before being transfected with the desired inhibitory or activating factors. Stem cells are undifferentiated biological cells that can differentiate into specialized cells and can divide to produce more stem cells. They are found in multicellular organisms. In adult organisms, stem cells and progenitor cells act as a repair system for the body, replenishing adult tissues. *Turritopsis nutricula* is a hydrozoan that can revert to the sexually immature (polyp stage) after becoming sexually mature. It is the only known metazoan capable of reverting completely to a sexually immature, colonial stage after having reached sexual maturity as a solitary stage. It does this through the cell development process of transdifferentiation. This cycle can repeat indefinitely tha offers it biologically immortal. It is not clear if stem cells are involved in this immortality or not (Wikipedia, 2015; Ma and Yang, 2010).

The following introduces recent reports as references in the related studies.

Ali, F. R., K. Cheng, et al. "The phosphorylation status of Ascl1 is a key determinant of neuronal differentiation and maturation in vivo and in vitro." Development **141**(11): 2216-24.

Generation of neurons from patient fibroblasts using a combination of developmentally defined transcription factors has great potential in disease modelling, as well as ultimately for use in regeneration and repair. However, generation of physiologically mature neurons in vitro remains problematic. Here we demonstrate the cell-cycle-dependent phosphorylation of a key reprogramming transcription factor, Ascl1, on multiple serine-proline sites. This multisite phosphorylation is a crucial regulator of the ability of Ascl1 to drive neuronal differentiation and maturation in vivo in the developing embryo; a phosphomutant form of Ascl1 shows substantially enhanced neuronal induction activity in Xenopus embryos. Mechanistically, we see that this un(der)phosphorylated Ascl1 is resistant to inhibition by both cyclin-dependent kinase activity and Notch signalling, both of which normally limit its neurogenic potential. Ascl1 is a central component of reprogramming transcription factor cocktails to generate neurons from human fibroblasts; the use of phosphomutant Ascl1 in place of the wild-type protein significantly promotes neuronal maturity after human fibroblast reprogramming in vitro. These results demonstrate that cell-cycle-dependent post-translational modification of proneural proteins directly regulates neuronal differentiation in vivo during development, and that this regulatory mechanism can be harnessed to promote maturation of neurons obtained by transdifferentiation of human cells in vitro.

Ansieau, S. "EMT in breast cancer stem cell generation." Cancer Lett **338**(1): 63-8.

The concept of cancer stem cells (CSCs) has been proposed to explain the ability of single disseminated cancer cells to reconstitute tumours with heterogeneity similar to that of the primary tumour they arise from. Although this concept is now commonly accepted, the origin of these CSCs remains a source of debate. First proposed to arise through stem/progenitor cell transformation, CSCs might also or alternatively arise from differentiated cancer cells through epithelial to mesenchymal transition (EMT), an embryonic transdifferentiation process. Using breast carcinomas as a study model, I propose revisiting the role of EMT in generating CSCs and the debate on potential underlying mechanisms and biological significance.

Bouwens, L., I. Houbracken, et al. "The use of stem cells for pancreatic regeneration in diabetes mellitus." Nat Rev Endocrinol **9**(10): 598-606.

The endocrine pancreas represents an interesting arena for regenerative medicine and cell therapeutics. One of the major pancreatic diseases, diabetes mellitus is a metabolic disorder caused by having an insufficient number of insulin-producing beta cells. Replenishment of beta cells by cell transplantation can restore normal metabolic control. The shortage in donor pancreata has meant that the demand for transplantable beta cells has outstripped the supply, which could be met by using alternative sources of stem cells. This situation has opened up new areas of research, such as cellular reprogramming and in vivo beta-cell regeneration. Pluripotent stem cells seem to be the best option for clinical applications of beta-cell regeneration in the near future, as these cells have been demonstrated to represent an unlimited source of functional beta cells. Although compelling evidence shows that the adult pancreas retains regenerative capacity, it remains unclear whether this organ contains stem cells. Alternatively, specialized cell types within or outside the pancreas retain plasticity in proliferation and differentiation. Cellular reprogramming or transdifferentiation of exocrine cells or other types of endocrine cells in the pancreas could provide a long-term solution.

Bronckaers, A., P. Hilkens, et al. "Mesenchymal stem/stromal cells as a pharmacological and therapeutic approach to accelerate angiogenesis." Pharmacol Ther **143**(2): 181-96.

Mesenchymal stem cells or multipotent stromal cells (MSCs) have initially captured attention in the scientific world because of their differentiation potential into osteoblasts, chondroblasts and adipocytes and possible transdifferentiation into neurons, glial cells and endothelial cells. This broad plasticity was originally hypothesized as the key mechanism of their demonstrated efficacy in numerous animal models of disease as well as in clinical settings. However, there is accumulating evidence suggesting that the beneficial effects of MSCs are predominantly caused by the multitude of bioactive molecules secreted by these remarkable cells. Numerous angiogenic factors, growth factors and cytokines have been discovered in the MSC secretome, all have been demonstrated to alter endothelial cell behavior in vitro and induce angiogenesis in vivo. As a consequence, MSCs have been widely explored as a promising treatment strategy in disorders caused by insufficient angiogenesis such as chronic wounds, stroke and myocardial infarction. In this review, we will summarize into detail the angiogenic factors found in the MSC secretome and their therapeutic mode of action in pathologies caused by limited blood vessel formation. Also the application of MSC as a vehicle to deliver drugs and/or genes in (anti-)angiogenesis will be discussed. Furthermore, the literature describing MSC transdifferentiation into endothelial cells will be evaluated critically.

Brzeszczynska, J., K. Samuel, et al. "Differentiation and molecular profiling of human embryonic stem cell-derived corneal epithelial cells." Int J Mol Med **33**(6): 1597-606.

It has been suggested that the isolation of scalable populations of limbal stem cells may lead to radical changes in ocular therapy. In particular, the derivation and transplantation of corneal stem cells from these populations may result in therapies providing clinical normality of the diseased or damaged cornea. Although feasible in theory, the lack of donor material in sufficient quantity and quality currently limits such a strategy. A potential scalable source of corneal cells could be derived from pluripotent stem cells (PSCs). We developed an in vitro and serum-free corneal differentiation model which displays significant promise. Our stepwise differentiation model was designed with reference to development and gave rise to cells which displayed similarities to epithelial progenitor cells which can be specified to cells displaying a corneal epithelial phenotype. We believe our approach is novel, provides a robust model of human development and in the future, may facilitate the generation of corneal epithelial cells that are suitable for clinical use. Additionally, we demonstrate that following continued cell culture, stem cell-derived corneal epithelial cells undergo transdifferentiation and exhibit squamous

metaplasia and therefore, also offer an in vitro model of disease.

Cassady, J. P., A. C. D'Alessio, et al. "Direct lineage conversion of adult mouse liver cells and B lymphocytes to neural stem cells." Stem Cell Reports **3**(6): 948-56.

Overexpression of transcription factors has been used to directly reprogram somatic cells into a range of other differentiated cell types, including multipotent neural stem cells (NSCs), that can be used to generate neurons and glia. However, the ability to maintain the NSC state independent of the inducing factors and the identity of the somatic donor cells remain two important unresolved issues in transdifferentiation. Here we used transduction of doxycycline-inducible transcription factors to generate stable tripotent NSCs. The induced NSCs (iNSCs) maintained their characteristics in the absence of exogenous factor expression and were transcriptionally, epigenetically, and functionally similar to primary brain-derived NSCs. Importantly, we also generated tripotent iNSCs from multiple adult cell types, including mature liver and B cells. Our results show that self-maintaining proliferative neural cells can be induced from nonectodermal cells by expressing specific combinations of transcription factors.

Chen, X., J. Fang, et al. "A new mosaic pattern in glioma vascularization: exogenous endothelial progenitor cells integrating into the vessels containing tumor-derived endothelial cells." Oncotarget **5**(7): 1955-68.

Emerging evidence suggests that glioma stem-like cells (GSCs) transdifferentiating into vascular endothelial cells (ECs) possibly contributes to tumor resistance to antiangiogenic therapy. Endothelial progenitor cells (EPCs), showing active migration and incorporation into neovasculature of glioma, may be a good vehicle for delivering genes to target GSCs transdifferentiation. Here, we found a new mosaic pattern that exogenous EPCs integrated into the vessels containing the tumor-derived ECs in C6 glioma rat model. Further, we evaluated the effect of these homing EPCs on C6 glioma cells transdifferentiation. The transdifferentiation frequency of C6 glioma cells and the expressions of key factors on GSCs transdifferentiation, i.e. HIF-1alpha, Notch1, and Flk1 in gliomas with or without EPCs transplantation showed no significant difference. Additionally, magnetic resonance imaging could track the migration and incorporation of EPCs into glioma in vivo, which was confirmed by Prussian blue staining. The number of magnetically labeled EPCs estimated from T2 maps correlated well with direct measurements of labeled cell counts by flow cytometry. Taken together, our findings may provide a rational base for the future application of EPCs as a therapeutic and imaging probe to overcome antiangiogenic resistance for glioma and monitor the efficacy of this treatment.

Chen, Y. S. and Z. P. Chen "Vasculogenic mimicry: a novel target for glioma therapy." Chin J Cancer **33**(2): 74-9.

Anti-angiogenic therapy has shown promising but insufficient efficacy on gliomas. Recent studies suggest that vasculogenic mimicry (VM), or the formation of non-endothelial, tumor-cell-lined microvascular channels, occurs in aggressive tumors, including gliomas. There is also evidence of a physiological connection between the endothelial-lined vasculature and VM channels. Tumor cells, by virtue of their high plasticity, can form vessel-like structures themselves, which may function as blood supply networks. Our previous study on gliomas showed that microvessel density was comparably less in VM-positive tumors than in VM-negative tumors. Thus, VM may act as a complement to ensure tumor blood supply, especially in regions with less microvessel density. Patients with VM-positive gliomas survived a shorter period of time than did patients with VM-negative gliomas. Although the detailed molecular mechanisms for VM are not fully understood, glioma stem cells might play a key role, since they are involved in tumor tissue remodeling and contribute to neovascularization via transdifferentiation. In the future, successful treatment of gliomas should involve targeting both VM and angiogenesis. In this review, we summarize the progress and challenges of VM in gliomas.

Cho, G. S., L. Fernandez, et al. "Regenerative medicine for the heart: perspectives on stem-cell therapy." Antioxid Redox Signal **21**(14): 2018-31.

SIGNIFICANCE: Despite decades of progress in cardiovascular biology and medicine, heart disease remains the leading cause of death, and there is no cure for the failing heart. Since heart failure is mostly caused by loss or dysfunction of cardiomyocytes (CMs), replacing dead or damaged CMs with new CMs might be an ideal way to reverse the disease. However, the adult heart is composed mainly of terminally differentiated CMs that have no significant self-regeneration capacity. RECENT ADVANCES: Stem cells have tremendous regenerative potential and, thus, current cardiac regenerative research has focused on developing stem cell sources to repair damaged myocardium. CRITICAL ISSUES: In this review, we examine the potential sources of cells that could be used for heart

therapies, including embryonic stem cells and induced pluripotent stem cells, as well as alternative methods for activating the endogenous regenerative mechanisms of the heart via transdifferentiation and cell reprogramming. We also discuss the current state of knowledge of cell purification, delivery, and retention. FUTURE DIRECTIONS: Efforts are underway to improve the current stem cell strategies and methodologies, which will accelerate the development of innovative stem-cell therapies for heart regeneration.

Chou, S. H., S. Z. Lin, et al. "Mesenchymal stem cell insights: prospects in cardiovascular therapy." Cell Transplant **23**(4-5): 513-29.

Ischemic heart damage usually triggers cardiomyopathological remodeling and fibrosis, thus promoting the development of heart functional failure. Mesenchymal stem cells (MSCs) are a heterogeneous group of cells in culture, with multipotent and hypoimmunogenic characters to aid tissue repair and avoid immune responses, respectively. Numerous experimental findings have proven the feasibility, safety, and efficiency of MSC therapy for cardiac regeneration. Despite that the exact mechanism remains unclear, the therapeutic ability of MSCs to treat ischemia heart diseases has been tested in phase I/II clinical trials. Based on encouraging preliminary findings, MSCs might become a potentially efficacious tool in the therapeutic options available to treat ischemic and nonischemic cardiovascular disorders. The molecular mechanism behind the efficacy of MSCs on promoting engraftment and accelerating the speed of heart functional recovery is still waiting for clarification. It is hypothesized that cardiomyocyte regeneration, paracrine mechanisms for cardiac repair, optimization of the niche for cell survival, and cardiac remodeling by inflammatory control are involved in the interaction between MSCs and the damaged myocardial environment. This review focuses on recent experimental and clinical findings related to cellular cardiomyoplasticity. We focus on MSCs, highlighting their roles in cardiac tissue repair, transdifferentiation, the MSC niche in myocardial tissues, discuss their therapeutic efficacy that has been tested for cardiac therapy, and the current bottleneck of MSC-based cardiac therapies.

Darabi, S., T. Tiraihi, et al. "Polarized neural stem cells derived from adult bone marrow stromal cells develop a rosette-like structure." In Vitro Cell Dev Biol Anim **49**(8): 638-52.

Bone marrow stromal cells (BMSCs) were reported to form floating aggregation of cells with expression of nestin, a marker for neural stem cells (NSCs). The purpose of this investigation is to evaluate the morphology and the molecular markers expressed by NSCs derived from these neurospheres. The BMSCs were isolated from Sprague Dawley rats and evaluated for osteogenesis, lipogenesis, and expression of fibronectin, CD90, CD106, CD31, and Oct4. The BMSCs were cultured with Dulbecco's modified Eagle's medium (DMEM)/F12 containing 15% fetal bovine serum, then with DMEM/F12 containing 2% B27, basic fibroblast growth factor, and epidermal growth factor. The cell aggregates or spheres were stained with acridine orange, which showed that the neurospheres comprised aggregated cells at either premitotic/postsynthetic (PS), postmitotic/presynthetic (PM) phases of cell cycle, or a mixture of both. The NSCs harvested from the neurospheres were polar with eccentric nucleus, and at either a PS or a PM cell cycle phases, some cells at the latter phase tended to form rosette-like structures. The cells were immunostained for molecular markers such as nestin, neurofilament 68 (NF68), NF160, and NF200 and glial fibrillary acidic protein (GFAP). Myelin basic protein (MBP), the pluripotency (Oct4, Nanog, and SOX2), and the differentiation genes (NeuroD1, Tubb4, and Musashi I) were also evaluated using reverse transcription polymerase chain reaction (RT-PCR). Nestin, NF68, NF160, NF200, GFAP, O4, and N-cadherin were expressed in the NSCs. The percentage of immunoreactive cells to nestin was significantly higher than that of the other neuronal markers. MBP was not expressed in BMSCs, neurospheres, and NSCs. The neurospheres were immunoreactive to GFAP. RT-PCR showed the expression of NeuroD1 and Musashi I. The pluripotency gene (SOX2) was expressed in NSCs. Oct4 and Nanog were expressed in BMSCs, while Oct4 and SOX2 were expressed in the neurosphere. This indicates that a pluripotency regularity network existed during the transdifferentiation of BMSCs into NSCs. Image processing of the neurospheres showed that the cells tended to form radial patterns. The conclusion of this study is that the NSCs generated from the BMSC-derived neurospheres have the morphology and the characteristics of neuroepithelial cells with tendency to forming rosette-like structures.

Derby, B. M., H. Dai, et al. "Adipose-derived stem cell to epithelial stem cell transdifferentiation: a mechanism to potentially improve understanding of fat grafting's impact on skin rejuvenation." Aesthet Surg J **34**(1): 142-53.

BACKGROUND: Recent evidence suggests that lipofilling improves overlying skin composition and appearance. Adipose-derived stem cells (ADSC) have been implicated. OBJECTIVE: The authors identify ADSC transdifferentiation into epithelial stem cells through coexpression of GFP+ (green fluorescent

protein positive) ADSC with the epithelial stem cell marker p63 in an in vivo fat grafting model. METHODS: Six male, GFP+ mice served as adipose tissue donors. Twelve nude mice served as recipients. Recipients were subdivided into 2 arms (6 mice/each arm) and received either whole-fat specimen (group 1) or isolated and purified ADSC + peptide hydrogel carrier (group 2) engrafted into a 1-cm(2) left parascapular subdermal plane. The right parascapular subdermal plane served as control. Skin flaps were harvested at 8 weeks and subjected to (1) confocal fluorescent microscopy and (2) reverse transcriptase polymerase chain reaction (RT-PCR) for p63 mRNA expression levels. RESULTS: Gross examination of skin flaps demonstrated subjectively increased dermal vessel presence surrounding whole-fat and ADSC specimens. The GFP+ cells were seen within overlying dermal architecture after engraftment and were found to coexpress p63. Significantly increased levels of p63 expression were found in the ADSC + hydrogel skin flaps. CONCLUSIONS: We offer suggestive evidence that GFP+ ADSC are found within the dermis 8 weeks after engraftment and coexpress the epithelial stem cell marker p63, indicating that ADSC may transdifferentiate into epithelial stem cells after fat grafting. These findings complement current understanding of how fat grafts may rejuvenate overlying skin.

Derynck, R., B. P. Muthusamy, et al. "Signaling pathway cooperation in TGF-beta-induced epithelial-mesenchymal transition." Curr Opin Cell Biol **31**: 56-66.

Transdifferentiation of epithelial cells into cells with mesenchymal properties and appearance, that is, epithelial-mesenchymal transition (EMT), is essential during development, and occurs in pathological contexts, such as in fibrosis and cancer progression. Although EMT can be induced by many extracellular ligands, TGF-beta and TGF-beta-related proteins have emerged as major inducers of this transdifferentiation process in development and cancer. Additionally, it is increasingly apparent that signaling pathways cooperate in the execution of EMT. This update summarizes the current knowledge of the coordination of TGF-beta-induced Smad and non-Smad signaling pathways in EMT, and the remarkable ability of Smads to cooperate with other transcription-directed signaling pathways in the control of gene reprogramming during EMT.

Dhanasekaran, M., S. Indumathi, et al. "Human omentum fat-derived mesenchymal stem cells transdifferentiates into pancreatic islet-like cluster." Cell Biochem Funct **31**(7): 612-9.

Current protocols of islet cell transplantation for the treatment of diabetes mellitus have been hampered by islet availability and allograft rejection. Although bone marrow and subcutaneous adipose tissue stem cells feature their tissue repair efficacy, applicability of stem cells from various sources is being researched to develop a promising therapy for diabetes mellitus. Although omentum fat has emerged as an innovative source of stem cells, the dearth of researches confirming its transdifferentiation potential limits its applicability as a regenerative tool in diabetic therapy. Thus, this work is a maiden attempt to explore the colossal potency of omentum fat-derived stem cells on its lucrative differentiation ability. The plasticity of omentum fat stem cells was substantiated by transdifferentiation into pancreatic islet-like clusters, which was confirmed by dithizone staining and immunocytochemistry for insulin. It was also confirmed by the expression of pancreatic endocrine markers nestin and pancreatic duodenal homeobox 1 (Pdx 1) using Fluorescence-activated cell sorting (FACS), neurogenic 3, islet-1 transcription factor, paired box gene 4, Pdx 1 and insulin using quantitative real-time polymerase chain reaction and through insulin secretion assay. This study revealed the in vitro differentiation potency of omentum fat stem cells into pancreatic islet-like clusters. However, further research pursuits exploring its in vivo endocrine efficacy would make omentum fat stem cells a superior source for beta-cell replacement therapy.

Di Stefano, B., J. L. Sardina, et al. "C/EBPalpha poises B cells for rapid reprogramming into induced pluripotent stem cells." Nature **506**(7487): 235-9.

CCAAT/enhancer binding protein-alpha (C/EBPalpha) induces transdifferentiation of B cells into macrophages at high efficiencies and enhances reprogramming into induced pluripotent stem (iPS) cells when co-expressed with the transcription factors Oct4 (Pou5f1), Sox2, Klf4 and Myc (hereafter called OSKM). However, how C/EBPalpha accomplishes these effects is unclear. Here we find that in mouse primary B cells transient C/EBPalpha expression followed by OSKM activation induces a 100-fold increase in iPS cell reprogramming efficiency, involving 95% of the population. During this conversion, pluripotency and epithelial-mesenchymal transition genes become markedly upregulated, and 60% of the cells express Oct4 within 2 days. C/EBPalpha acts as a 'path-breaker' as it transiently makes the chromatin of pluripotency genes more accessible to DNase I. C/EBPalpha also induces the expression of the dioxygenase Tet2 and promotes its translocation to the nucleus where it binds to regulatory regions of pluripotency genes that become demethylated after OSKM induction. In line with these

findings, overexpression of Tet2 enhances OSKM-induced B-cell reprogramming. Because the enzyme is also required for efficient C/EBPalpha-induced immune cell conversion, our data indicate that Tet2 provides a mechanistic link between iPS cell reprogramming and B-cell transdifferentiation. The rapid iPS reprogramming approach described here should help to fully elucidate the process and has potential clinical applications.

Duke, C. M. and H. S. Taylor "Stem cells and the reproductive system: historical perspective and future directions." Maturitas **76**(3): 284-9.

Recent findings in stem cell biology have presented new perspectives and opportunities for the treatment of reproductive disease. In a departure from the long held dogma of embryologically fixed numbers of oocytes, current literature suggests that human ovaries contain stem cells which form new oocytes even in adulthood and that these stem cells can be cultured in vitro to develop into mature oocytes. These findings have provided new hope and broader options for fertility preservation. Evidence of endometrial regeneration by bone marrow stem cells in endometrial tissue of women who received bone marrow transplant highlight potential for the novel treatments of uterine disorders and supports new theories for the etiology of endometriosis - ectopic transdifferentiation of stem cells. Further, endometrial derived stem cells have been demonstrated to be useful in the treatment of several chronic and often debilitating diseases, including Parkinson's Disease and Diabetes. Other cells that may present future therapeutic benefits for a myriad of disease states include placental and fetal cells which enter maternal circulation during pregnancy and can later promote parenchymal regeneration in maternal tissue. These findings highlight novel functions of the uterus and ovaries. They demonstrate that the uterus is a dynamic organ permeable to fetal stem cells capable of transdifferentiation as well as a renewable source of multipotent stem cells. While we still have much to understand about stem cells, their potential applications in reproductive biology and medicine are countless.

Dzafic, E., M. Stimpfel, et al. "Plasticity of granulosa cells: on the crossroad of stemness and transdifferentiation potential." J Assist Reprod Genet **30**(10): 1255-61.

The ovarian follicle represents the basic functional unit of the ovary and consists of an oocyte, which is surrounded by granulosa cells (GCs). GCs play an important role in the growth and development of the follicle. They are subject to increased attention since it has recently been shown that the subpopulation of GCs within the growing follicle possesses exceptionally plasticity showing stem cell characteristics. In assisted reproduction programs, oocytes are retrieved from patients together with GCs, which are currently discarded daily, but could be an interesting subject to be researched and potentially used in regenerative medicine in the future. Isolated GCs expressed stem cell markers such as OCT-4, NANOG and SOX-2, showed high telomerase activity, and were in vitro differentiated into other cell types, otherwise not present within ovarian follicles. Recently another phenomenon demonstrated in GCs is transdifferentiation, which could explain many ovarian pathological conditions. Possible applications in regenerative medicine are also given.

Espin-Palazon, R., D. L. Stachura, et al. "Proinflammatory signaling regulates hematopoietic stem cell emergence." Cell **159**(5): 1070-85.

Hematopoietic stem cells (HSCs) underlie the production of blood and immune cells for the lifetime of an organism. In vertebrate embryos, HSCs arise from the unique transdifferentiation of hemogenic endothelium comprising the floor of the dorsal aorta during a brief developmental window. To date, this process has not been replicated in vitro from pluripotent precursors, partly because the full complement of required signaling inputs remains to be determined. Here, we show that TNFR2 via TNF? activates the Notch and NF-?B signaling pathways to establish HSC fate, indicating a requirement for inflammatory signaling in HSC generation. We determine that primitive neutrophils are the major source of TNF?, assigning a role for transient innate immune cells in establishing the HSC program. These results demonstrate that proinflammatory signaling, in the absence of infection, is utilized by the developing embryo to generate the lineal precursors of the adult hematopoietic system.

Fouraschen, S. M., S. R. Hall, et al. "Support of hepatic regeneration by trophic factors from liver-derived mesenchymal stromal/stem cells." Methods Mol Biol **1213**: 89-104.

Mesenchymal stromal/stem cells (MSCs) have multilineage differentiation potential and as such are known to promote regeneration in response to tissue injury. However, accumulating evidence indicates that the regenerative capacity of MSCs is not via transdifferentiation but mediated by their production of trophic and other factors that promote endogenous regeneration pathways of the tissue cells. In this chapter, we provide a detailed description on how to obtain trophic factors secreted by cultured MSCs and how they can be used in small animal models. More specific, in vivo models to study the

paracrine effects of MSCs on regeneration of the liver after surgical resection and/or ischemia and reperfusion injury are described.

Fu, L., X. Zhu, et al. "Regenerative medicine: transdifferentiation in vivo." Cell Res **24**(2): 141-2.

A major challenge in regenerative medicine is the generation of functionally effective target cells to replace or repair damaged tissues. Transdifferentiation in vivo is a novel strategy to achieve cell fate conversion within the native physiological niche; this technology may provide a time- and cost-effective alternative for applications in regenerative medicine and may also minimize the concerns associated with in vitro culture and cell transplantation.

Fuhrmann, S., C. Zou, et al. "Retinal pigment epithelium development, plasticity, and tissue homeostasis." Exp Eye Res **123**: 141-50.

The retinal pigment epithelium (RPE) is a simple epithelium interposed between the neural retina and the choroid. Although only 1 cell-layer in thickness, the RPE is a virtual workhorse, acting in several capacities that are essential for visual function and preserving the structural and physiological integrities of neighboring tissues. Defects in RPE function, whether through chronic dysfunction or age-related decline, are associated with retinal degenerative diseases including age-related macular degeneration. As such, investigations are focused on developing techniques to replace RPE through stem cell-based methods, motivated primarily because of the seemingly limited regeneration or self-repair properties of mature RPE. Despite this, RPE cells have an unusual capacity to transdifferentiate into various cell types, with the particular fate choices being highly context-dependent. In this review, we describe recent findings elucidating the mechanisms and steps of RPE development and propose a developmental framework for understanding the apparent contradiction in the capacity for low self-repair versus high transdifferentiation.

Gamez Escalona, J. A. and N. Lopez Moratalla "[Pluripotent stem cells on cell therapy]." An Sist Sanit Navar **37**(1): 129-36.

Induced pluripotent stem (iPS) cells are a novel stem cell population derived from human somatic cells through reprogramming using a set of transcription factors. These iPS cells were shown to share the characteristics of embryonic stem cells, including the ability to give rise to differentiated cells of every tissue type of the body. In the shorter term, iPS cells will be useful for creating patient-identical disease model cells in which the pathological process can be studied and drugs can be tested. Despite critical attitudes, accumulating preclinical evidence supports the effectiveness of iPSC-based cell therapy on the selection of appropriate iPSC clones. The production of iPS cells has also spurred the development of other techniques, for example, transdifferentiation by researchers can now convert heart fibroblasts directly in vivo into myocytes by similar methods. This pluripotent cells is indeed of great value in medical research and it is opening new possibilities in cell therapy.

Gehmert, S., C. Wenzel, et al. "Adipose tissue-derived stem cell secreted IGF-1 protects myoblasts from the negative effect of myostatin." Biomed Res Int **2014**: 129048.

Myostatin, a TGF-beta family member, is associated with inhibition of muscle growth and differentiation and might interact with the IGF-1 signaling pathway. Since IGF-1 is secreted at a bioactive level by adipose tissue-derived mesenchymal stem cells (ASCs), these cells (ASCs) provide a therapeutic option for Duchenne Muscular Dystrophy (DMD). But the protective effect of stem cell secreted IGF-1 on myoblast under high level of myostatin remains unclear. In the present study murine myoblasts were exposed to myostatin under presence of ASCs conditioned medium and investigated for proliferation and apoptosis. The protective effect of IGF-1 was further examined by using IGF-1 neutralizing and receptor antibodies as well as gene silencing RNAi technology. MyoD expression was detected to identify impact of IGF-1 on myoblasts differentiation when exposed to myostatin. IGF-1 was accountable for 43.6% of the antiapoptotic impact and 48.8% for the proliferative effect of ASCs conditioned medium. Furthermore, IGF-1 restored mRNA and protein MyoD expression of myoblasts under risk. Beside fusion and transdifferentiation the beneficial effect of ASCs is mediated by paracrine secreted cytokines, particularly IGF-1. The present study underlines the potential of ASCs as a therapeutic option for Duchenne muscular dystrophy and other dystrophic muscle diseases.

Ghasemzadeh-Hasankolaei, M., M. A. Sedighi-Gilani, et al. "Induction of ram bone marrow mesenchymal stem cells into germ cell lineage using transforming growth factor-beta superfamily growth factors." Reprod Domest Anim **49**(4): 588-98.

Several studies have proposed that in vitro generation of germ cells (GCs) from stem cells can be considered a future option for infertility treatment. Mesenchymal stem cells (MSCs) have the capability to differentiate into male GCs with the use of inducers such as retinoic acid. Transforming growth factor-beta 1 (TGFb1) has been shown to play important roles in

male fertility and spermatogenesis. Bone morphogenic protein 4 (BMP4) and BMP8b are also involved in the derivation of primordial GCs (PGCs) from epiblast cells. Therefore, this study aims to determine whether TGFb1, BMP4 and BMP8b can initiate transdifferentiation of MSCs into GCs in vitro and to determine the type of changes that occur in the expression of GC-specific markers. In this study, we have divided passage-3 ram bone marrow (BM)-MSCs into three main groups (BMP4, BMP8b and TGFb1) which were separately treated with 10 ng/ml TGFb1, 100 ng/ml BMP4 and 100 ng/ml BMP8b for a period of 21 days. We have evaluated the ability of these groups to differentiate into GCs by assessing expressions of GC-specific markers with reverse transcription PCR (RT-PCR), quantitative RT-PCR (qRT-PCR), immunocytochemistry, morphological changes and alkaline phosphatase (ALP) activity. Our results showed that BMP4 and BMP8b induced PGCs properties in some BM-MSCs and TGFb1 formed spermatogonial stem cells (SSCs) and spermatogonia-like cells in BM-MSCs culture. The important results of this study provide the basis for additional studies to determine the exact mechanism of GCs differentiation and possibly solve the problem of infertility.

Giordano, A., A. Smorlesi, et al. "White, brown and pink adipocytes: the extraordinary plasticity of the adipose organ." Eur J Endocrinol **170**(5): R159-71.

In mammals, adipocytes are lipid-laden cells making up the parenchyma of the multi-depot adipose organ. White adipocytes store lipids for release as free fatty acids during fasting periods; brown adipocytes burn glucose and lipids to maintain thermal homeostasis. A third type of adipocyte, the pink adipocyte, has recently been characterised in mouse subcutaneous fat depots during pregnancy and lactation. Pink adipocytes are mammary gland alveolar epithelial cells whose role is to produce and secrete milk. Emerging evidence suggests that they derive from the transdifferentiation of subcutaneous white adipocytes. The functional response of the adipose organ to a range of metabolic and environmental challenges highlights its extraordinary plasticity. Cold exposure induces an increase in the 'brown' component of the organ to meet the increased thermal demand; in states of positive energy balance, the 'white' component expands to store excess nutrients; finally, the 'pink' component develops in subcutaneous depots during pregnancy to ensure litter feeding. At the cell level, plasticity is provided not only by stem cell proliferation and differentiation but also, distinctively, by direct transdifferentiation of fully differentiated adipocytes by the stimuli that induce genetic expression reprogramming and through it a change in phenotype and, consequently function. A greater understanding of adipocyte transdifferentiation mechanisms would have the potential to shed light on their biology as well as inspire novel therapeutic strategies against metabolic syndrome (browning) and breast cancer (pinking).

Guo, J., H. Wang, et al. "Reprogramming and transdifferentiation shift the landscape of regenerative medicine." DNA Cell Biol **32**(10): 565-72.

Regenerative medicine is a new interdisciplinary field in biomedical science, which aims at the repair or replacement of the defective tissue or organ by congenital defects, age, injury, or disease. Various cell-related techniques such as stem cell-based biotherapy are a hot topic in the current press, and stem cell research can help us to expand our understanding of development as well as the pathogenesis of disease. In addition, new technology such as reprogramming or dedifferentiation and transdifferentiation open a new area for regenerative medicine. Here we review new approaches of these technologies used for cell-based therapy and discuss future directions and challenges in the field of regeneration.

Harkin, D. G., L. Foyn, et al. "Concise reviews: can mesenchymal stromal cells differentiate into corneal cells? A systematic review of published data." Stem Cells **33**(3): 785-91.

The majority of stem cell therapies for corneal repair are based upon the use of progenitor cells isolated from corneal tissue, but a growing body of literature suggests a role for mesenchymal stromal cells (MSC) isolated from noncorneal tissues. While the mechanism of MSC action seems likely to involve their immuno-modulatory properties, claims have emerged of MSC transdifferentiation into corneal cells. Substantial differences in methodology and experimental outcomes, however, have prompted us to perform a systematic review of the published data. Key questions used in our analysis included: the choice of markers used to assess corneal cell phenotype, the techniques used to detect these markers, adequate reporting of controls, and tracking of MSC when studied in vivo. Our search of the literature revealed 28 papers published since 2006, with half appearing since 2012. MSC cultures established from bone marrow and adipose tissue have been best studied (22 papers). Critically, only 11 studies used appropriate markers of corneal cell phenotype, along with necessary controls. Ten out of these eleven papers, however, contained positive evidence of corneal cell marker expression by MSC. The clearest evidence is observed with respect to expression of markers for corneal stromal cells by MSC. In comparison, the evidence for MSC

conversion into either corneal epithelial cells or corneal endothelial cells is often inconsistent or inconclusive. Our analysis clarifies this emerging body of literature and provides guidance for future studies of MSC differentiation within the cornea as well as other tissues. Stem Cells 2015;33:785-791.

Hodgetts, S. I., P. J. Simmons, et al. "A comparison of the behavioral and anatomical outcomes in sub-acute and chronic spinal cord injury models following treatment with human mesenchymal precursor cell transplantation and recombinant decorin." Exp Neurol **248**: 343-59.

This study assessed the potential of highly purified (Stro-1(+)) human mesenchymal precursor cells (hMPCs) in combination with the anti-scarring protein decorin to repair the injured spinal cord (SC). Donor hMPCs isolated from spinal cord injury (SCI) patients were transplanted into athymic rats as a suspension graft, alone or after previous treatment with, core (decorin(core)) and proteoglycan (decorin(pro)) isoforms of purified human recombinant decorin. Decorin was delivered via mini-osmotic pumps for 14 days following sub-acute (7 day) or chronic (1 month) SCI. hMPCs were delivered to the spinal cord at 3 weeks or 6 weeks after the initial injury at T9 level. Behavioral and anatomical analysis in this study showed statistically significant improvement in functional recovery, tissue sparing and cyst volume reduction following hMPC therapy. The combination of decorin infusion followed by hMPC therapy did not improve these measured outcomes over the use of cell therapy alone, in either sub-acute or chronic SCI regimes. However, decorin infusion did improve tissue sparing, reduce spinal tissue cavitation and increase transplanted cell survivability as compared to controls. Immunohistochemical analysis of spinal cord sections revealed differences in glial, neuronal and extracellular matrix molecule expression within each experimental group. hMPC transplanted spinal cords showed the increased presence of serotonergic (5-HT) and sensory (CGRP) axonal growth within and surrounding transplanted hMPCs for up to 2 months; however, no evidence of hMPC transdifferentiation into neuronal or glial phenotypes. The number of hMPCs was dramatically reduced overall, and no transplanted cells were detected at 8 weeks post-injection using lentiviral GFP labeling and human nuclear antigen antibody labeling. The presence of recombinant decorin in the cell transplantation regimes delayed in part the loss of donor cells, with small numbers remaining at 2 months after transplantation. In vitro co-culture experiments with embryonic dorsal root ganglion explants revealed the growth promoting properties of hMPCs. Decorin did not increase axonal outgrowth from that achieved by hMPCs. We provide evidence for the first time that (Stro-1(+)) hMPCs provide: i) an advantageous source of allografts for stem cell transplantation for sub-acute and chronic spinal cord therapy, and (ii) a positive host microenvironment that promotes tissue sparing/repair that subsequently improves behavioral outcomes after SCI. This was not measurably improved by recombinant decorin treatment, but does provide important information for the future development and potential use of decorin in contusive SCI therapy.

Huang, X. G., Y. Z. Chen, et al. "Rac1 modulates the vitreous-induced plasticity of mesenchymal movement in retinal pigment epithelial cells." Clin Experiment Ophthalmol **41**(8): 779-87.

BACKGROUND: The vitreous has been shown to induce epithelial-mesenchymal transdifferentiation because it induces fibroblast-like morphology, enhanced migration and invasion in retinal pigment epithelial cells in proliferative vitreoretinopathy. Rac1 is the principal mediator of cell migration. In the current study, the relationship between Rac1 and cell migration, and invasion in vitreous-transformed retinal pigment epithelial cells was investigated using NSC23766, a specific inhibitor of Rac guanosine-5'-triphosphatase activity, and the involvement of a Rac1 guanosine-5'-triphosphatase-dependent pathway was detected. DESIGN: One-way design with multiple levels and repeated measurement design. PARTICIPANTS AND SAMPLES: The vitreous humor was collected from 20 healthy donor eyes and the retinal pigment epithelial cells were obtained from 9 healthy donor eyes. METHODS: Human low-passage retinal pigment epithelial cells were treated with normal medium or 25% vitreous medium. Rac1 activity was measured using a pull-down assay. The cytotoxicity of NSC23766 was measured using the trypan blue dye exclusion test. Cell migration was measured using a wound healing assay. Cell invasion was determined using a transwell invasion assay. Protein expression of Rac1 and phosphorylation of LIM kinase 1 and cofilin were detected by Western blot analysis. MAIN OUTCOME MEASURES: Cell migration, invasion, Rac1 activity and phosphorylation of LIM kinase 1 and cofilin. RESULTS: Rac1guanosine-5'-triphosphatase was activated in vitreous-transformed retinal pigment epithelial cells. A Rac inhibitor suppressed vitreous-induced migration and invasion in retinal pigment epithelial cells. Cofilin phosphorylation was activated by vitreous treatment but blocked by NSC23766. CONCLUSIONS: Rac1 mediates vitreous-transformed retinal pigment epithelial cells' plasticity of mesenchymal movement via Rac1 guanosine-5'-triphosphatase-dependent pathways that modulate LIM kinase 1 and cofilin activity. Rac inhibition may be

considered a novel treatment for proliferative vitreoretinopathy.

Isik, S., M. Zaim, et al. "DNA topoisomerase IIbeta as a molecular switch in neural differentiation of mesenchymal stem cells." Ann Hematol **94**(2): 307-18.

Two isoforms of DNA topoisomerase II (topo II) have been identified in mammalian cells, named topo IIalpha and topo IIbeta. Topo IIalpha plays an essential role in segregation of daughter chromosomes and thus for cell proliferation in mammalian cells. Unlike its isozyme topo IIalpha, topo IIbeta is greatly expressed upon terminal differentiation of neuronal cells. Although there have been accumulating evidence about the crucial role of topo IIbeta in neural development through activation or repression of developmentally regulated genes at late stages of neuronal differentiation, there have been no reports that analyzed the roles of topo IIbeta in the neural trans differentiation process of multipotent stem cells. Terminal differentiation of neurons and transdifferentiation of Mesenchymal Stem Cells (MSCs) are two distinct processes. Therefore, the functional significance of topo IIbeta may also be different in these differentiation systems. MSC transdifferentiation into neuron-like cells represents an useful model to further validate the role of topo IIbeta in neuronal differentiation. The aim of this study is to evaluate the subset of genes that are regulated in neural transdifferentiation of bone marrow-derived human MSCs (BM-hMSCs) in vitro and find genes related with topo IIbeta. For this purpose, topo IIbeta was silenced by specific small interfering RNAs in hMSCs and cells were induced to differentiate into neuron-like cells. Differentiation and silencing of topo IIbeta were monitored by real-time cell analysis and also expressions of topo II isoforms were analyzed. Change in transcription patterns of genes upon topo IIbeta silencing was identified by DNA microarray analysis, and apparently genes involved in regulation of several ion channels and transporters, vesicle function, and cell calcium metabolism were particularly affected by topo IIbeta silencing suggesting that topoIIbeta silencing can significantly alter the gene expression pattern of genes involved in variety of biological processes and signal transduction pathways including transcription, translation, cell trafficking, vesicle function, transport, cell morphology, neuron guidance, growth, polarity, and axonal growth. It appears that the deregulation of these pathways may contribute to clarify the further role of topo IIbeta in neural differentiation.

Israely, E., M. Ginsberg, et al. "Akt suppression of TGFbeta signaling contributes to the maintenance of vascular identity in embryonic stem cell-derived endothelial cells." Stem Cells **32**(1): 177-90.

The ability to generate and maintain stable in vitro cultures of mouse endothelial cells (ECs) has great potential for genetic dissection of the numerous pathologies involving vascular dysfunction as well as therapeutic applications. However, previous efforts at achieving sustained cultures of primary stable murine vascular cells have fallen short, and the cellular requirements for EC maintenance in vitro remain undefined. In this study, we have generated vascular ECs from mouse embryonic stem (ES) cells and show that active Akt is essential to their survival and propagation as homogeneous monolayers in vitro. These cells harbor the phenotypical, biochemical, and functional characteristics of ECs and expand throughout long-term cultures, while maintaining their angiogenic capacity. Moreover, Akt-transduced embryonic ECs form functional perfused vessels in vivo that anastomose with host blood vessels. We provide evidence for a novel function of Akt in stabilizing EC identity, whereby the activated form of the protein protects mouse ES cell-derived ECs from TGFbeta-mediated transdifferentiation by downregulating SMAD3. These findings identify a role for Akt in regulating the developmental potential of ES cell-derived ECs and demonstrate that active Akt maintains endothelial identity in embryonic ECs by interfering with active TGFbeta-mediated processes that would ordinarily usher these cells to alternate fates.

Jung, D. W., Y. J. Hong, et al. "5-Nitro-5'hydroxy-indirubin-3'oxime is a novel inducer of somatic cell transdifferentiation." Arch Pharm (Weinheim) **347**(11): 806-18.

Patient-derived cell transplantation is an attractive therapy for regenerative medicine. However, this requires effective strategies to reliably differentiate patient cells into clinically useful cell types. Herein, we report the discovery that 5-nitro-5'hydroxy-indirubin-3'oxime (5'-HNIO) is a novel inducer of cell transdifferentiation. 5'-HNIO induced muscle transdifferentiation into adipogenic and osteogenic cells. 5'-HNIO was shown to inhibit aurora kinase A, which is a known cell fate regulator. 5'-HNIO produced a favorable level of transdifferentiation compared to other aurora kinase inhibitors and induced transdifferentiation across cell lineage boundaries. Significantly, 5'-HNIO treatment produced direct transdifferentiation without up-regulating potentially oncogenic induced pluripotent stem cell (iPSC) reprogramming factors. Thus, our results demonstrate that 5'-HNIO is an attractive molecular tool for cell transdifferentiation and cell fate research.

Jung, D. W., W. H. Kim, et al. "Reprogram or reboot: small molecule approaches for the production of induced pluripotent stem cells and direct cell reprogramming." ACS Chem Biol **9**(1): 80-95.

Stem cell transplantation is a potential therapy for regenerative medicine, which aims to restore tissues damaged by trauma, aging, and diseases. Since its conception in the late 1990s, chemical biology has provided powerful and diverse small molecule tools for modulating stem cell function. Embryonic stem cells could be an ideal source for transplantation, but ethical concerns restrict their development for cell therapy. The seminal advance of induced pluripotent stem cell (iPSC) technology provided an attractive alternative to human embryonic stem cells. However, iPSCs are not yet considered an ideal stem cell source, due to limitations associated with the reprogramming process and their potential tumorigenic behavior. This is an area of research where chemical biology has made a significant contribution to facilitate the efficient production of high quality iPSCs and elucidate the biological mechanisms governing their phenotype. In this review, we summarize these advances and discuss the latest progress in developing small molecule modulators. Moreover, we also review a new trend in stem cell research, which is the direct reprogramming of readily accessible cell types into clinically useful cells, such as neurons and cardiac cells. This is a research area where chemical biology is making a pivotal contribution and illustrates the many advantages of using small molecules in stem cell research.

Kabara, M., J. Kawabe, et al. "Immortalized multipotent pericytes derived from the vasa vasorum in the injured vasculature. A cellular tool for studies of vascular remodeling and regeneration." Lab Invest **94**(12): 1340-54.

Adventitial microvessels, vasa vasorum in the vessel walls, have an active role in the vascular remodeling, although its mechanisms are still unclear. It has been reported that microvascular pericytes (PCs) possess mesenchymal plasticity. Therefore, microvessels would serve as a systemic reservoir of stem cells and contribute to the tissues remodeling. However, most aspects of the biology of multipotent PCs (mPCs), in particular of pathological microvessels are still obscure because of the lack of appropriate methods to detect and isolate these cells. In order to examine the characteristics of mPCs, we established immortalized cells residing in adventitial capillary growing at the injured vascular walls. We recently developed in vivo angiogenesis to observe adventitial microvessels using collagen-coated tube (CCT), which also can be used as an adventitial microvessel-rich tissue. By using the CCT, CD146- or NG2-positive cells were isolated from the adventitial microvessels in the injured arteries of mice harboring a temperature-sensitive SV40 T-antigen gene. Several capillary-derived endothelial cells (cECs) and PCs (cPCs) cell lines were established. cECs and cPCs maintain a number of key endothelial and PC features. Co-incubation of cPCs with cECs formed capillary-like structure in Matrigel. Three out of six cPC lines, termed capillary mPCs demonstrated both mesenchymal stem cell- and neuronal stem cell-like phenotypes, differentiating effectively into adipocytes, osteoblasts, as well as schwann cells. mPCs differentiated to ECs and PCs, and formed capillary-like structure on their own. Transplanted DsRed-expressing mPCs were resident in the capillary and muscle fibers and promoted angiogenesis and myogenesis in damaged skeletal muscle. Adventitial mPCs possess transdifferentiation potential with unique phenotypes, including the reconstitution of capillary-like structures. Their phenotype would contribute to the pathological angiogenesis associated with vascular remodeling. These cell lines also provide a reproducible cellular tool for high-throughput studies on angiogenesis, vascular remodeling, and regeneration as well.

Katz, L. S., E. Geras-Raaka, et al. "Reprogramming adult human dermal fibroblasts to islet-like cells by epigenetic modification coupled to transcription factor modulation." Stem Cells Dev **22**(18): 2551-60.

In this article, we describe novel conditions for culture, expansion, and transdifferentiation of primary human dermal fibroblasts (hDFs) to induce expression of transcription factors (TFs) and hormones characteristic of the islets of Langerhans. We show that histones associated with the insulin gene are hyperacetylated and that insulin gene DNA is less methylated in islet cells compared to cells that do not express insulin. Using two compounds that alter the epigenetic signature of cells, romidepsin (Romi), a histone deacetylase inhibitor, and 5-Azacytidine (5-AzC), a chemical analogue of cytidine that cannot be methylated, we show that hDFs exhibit a distinctive regulation of expression of TFs involved in islet development as well as of induction of glucagon and insulin. Overexpression of Pdx1, a TF important for islet differentiation, and silencing of musculoaponeurotic fibrosarcoma oncogene homolog B, a TF that is expressed in mature glucagon-producing cells, result in induction of insulin to a higher level compared to Romi and 5-AzC alone. The cells obtained from this protocol exhibit glucose-stimulated insulin secretion and lower blood glucose levels of diabetic mice. These data show that fully

differentiated nonislet-derived cells could be made to transdifferentiate to islet-like cells and that combining epigenetic modulation with TF modulation leads to enhanced insulin expression.

Kaur, K., J. Yang, et al. "5-azacytidine promotes the transdifferentiation of cardiac cells to skeletal myocytes." Cell Reprogram **16**(5): 324-30.

The DNA methylation inhibitor 5-azacytidine is widely used to stimulate the cardiac differentiation of stem cells. However, 5-azacytidine has long been employed as a tool for stimulating skeletal myogenesis. Yet, it is unclear whether the ability of 5-azacytidine to promote both cardiac and skeletal myogenesis is dependent strictly on the native potential of the starting cell population or if this drug is a transdifferentiation agent. To address this issue, we examined the effect of 5-azacytidine on cultures of adult mouse atrial tissue, which contains cardiac but not skeletal muscle progenitors. Exposure to 5-azacytidine caused atrial cells to elongate and increased the presence of fat globules within the cultures. 5-Azacytidine also induced expression of the skeletal myogenic transcription factors MyoD and myogenin. 5-Azacytidine pretreatments allowed atrial cells to undergo adipogenesis or skeletal myogenesis when subsequently cultured with either insulin and dexamethasone or low-serum media, respectively. The presence of skeletal myocytes in atrial cultures was indicated by dual staining for myogenin and sarcomeric alpha-actin. These data demonstrate that 5-azacytidine converts cardiac cells to noncardiac cell types and suggests that this drug has a compromised efficacy as a cardiac differentiation factor.

Kelaini, S., A. Cochrane, et al. "Direct reprogramming of adult cells: avoiding the pluripotent state." Stem Cells Cloning **7**: 19-29.

The procedure of using mature, fully differentiated cells and inducing them toward other cell types while bypassing an intermediate pluripotent state is termed direct reprogramming. Avoiding the pluripotent stage during cellular conversions can be achieved either through ectopic expression of lineage-specific factors (transdifferentiation) or a direct reprogramming process that involves partial reprogramming toward the pluripotent stage. Latest advances in the field seek to alleviate concerns that include teratoma formation or retroviral usage when it comes to delivering reprogramming factors to cells. They also seek to improve efficacy and efficiency of cellular conversion, both in vitro and in vivo. The final products of this reprogramming approach could be then directly implemented in regenerative and personalized medicine.

Kikuchi, K. "Dedifferentiation, Transdifferentiation, and Proliferation: Mechanisms Underlying Cardiac Muscle Regeneration in Zebrafish." Curr Pathobiol Rep **3**(1): 81-88.

The adult mammalian heart is increasingly recognized as a regenerative organ with a measurable capacity to replenish cardiomyocytes throughout its lifetime, illuminating the possibility of stimulating endogenous regenerative capacity to treat heart diseases. Unlike mammals, certain vertebrates possess robust capacity for regenerating a damaged heart, providing a model to understand how regeneration could be augmented in injured human hearts. Facilitated by its rich history in the study of heart development, the teleost zebrafish Danio rerio has been established as a robust model to investigate the underlying mechanism of cardiac regeneration. This review discusses the current understanding of the endogenous mechanisms behind cardiac regeneration in zebrafish, with a particular focus on cardiomyocyte dedifferentiation, transdifferentiation, and proliferation.

Kim, J. and J. Ko "A novel PPARgamma2 modulator sLZIP controls the balance between adipogenesis and osteogenesis during mesenchymal stem cell differentiation." Cell Death Differ **21**(10): 1642-55.

Mesenchymal stem cells (MSCs), also known as multipotent stromal cells, are used in clinical trials. However, the use of MSCs for medical treatment of patients poses a potential problem due to the possibility of transdifferentiation into unwanted tissues. Disruption of the balance during MSC differentiation leads to obesity, skeletal fragility, and osteoporosis. Differentiation of MSCs into either adipocytes or osteoblasts is transcriptionally regulated by the two key transcription factors PPARgamma2 and Runx2. PPARgamma2 is highly expressed during adipocyte differentiation and regulates expression of genes involved in adipogenesis. Runx2 induces osteogenic gene expression and, thereby, increases osteoblast differentiation. Although transcriptional modulation of PPARgamma2 has been investigated in adipogenesis, the underlying molecular mechanisms to control the balance between adipogenesis and osteogenesis in MSCs remain unclear. In this study, the role of sLZIP in regulation of PPARgamma2 transcriptional activation was investigated along with sLZIP's involvement in differentiation of MSCs into adipocytes and osteoblasts. sLZIP interacts with PPARgamma2 and functions as a corepressor of PPARgamma2. sLZIP enhances formation of the PPARgamma2 corepressor complex through specific interaction with HDAC3, resulting in suppression of PPARgamma2 transcriptional activity. We found that sLZIP prevents expression of PPARgamma2 target

genes and adipocyte differentiation both in vitro and in vivo. sLZIP also upregulates Runx2 transcriptional activity via inhibition of PPARgamma2 activity, and promotes osteoblast differentiation. sLZIP transgenic mice exhibited enhanced bone mass and density, compared with wild-type mice. These results indicate that sLZIP has a critical role in the regulation of osteogenesis and bone development. However, sLZIP does not affect chondrogenesis and osteoclastogenesis. We propose that sLZIP is a novel PPARgamma2 modulator for control of the balance between adipogenesis and osteogenesis during MSC differentiation, and that sLZIP can be used as a therapeutic target molecule for treatment of obesity, osteodystrophy, and osteoporosis.

Kim, J. W., S. Y. Park, et al. "Targeting PGC-1alpha to overcome the harmful effects of glucocorticoids in porcine neonatal pancreas cell clusters." Transplantation **97**(3): 273-9.

BACKGROUND: Peroxisome proliferator-activated receptor gamma-coactivator-1alpha (PGC-1alpha) has recently been implicated as a crucial factor in the glucocorticoid-suppressed expansion and transdifferentiation of porcine neonatal pancreatic cell clusters (NPCCs). However, the molecular mechanism has not been clarified. METHODS: We investigated whether the suppression of PGC-1alpha expression protects against beta-cell dysfunction induced by dexamethasone (Dx) treatment in vitro and in vivo and determined the mechanism of action of PGC-1alpha in porcine NPCCs. RESULTS: The reduction in Pdx-1 gene expression caused by either Dx treatment or PGC-1alpha overexpression was normalized by siPGC-1alpha. Nuclear FOXO1 and cytoplasmic Pdx-1 were detected after Dx treatment. However, FOXO1 was observed in the cytoplasm, and Pdx-1 was observed in the nucleus after siPGC-1alpha. Suppression of PGC-1alpha by siPGC-1alpha improved the Dx-induced repression of insulin secretion and insulin content. In vivo studies showed that the glucose level in the Ad-siPGC-1alpha-infected group was significantly lower than that in the Dx-treated group. Insulin expression in the graft tissue disappeared in the Dx-injected group. However, the siPGC-1alpha- and Dx-treated group showed increased insulin expression and an increase in graft mass, beta-cell mass, and beta-cell % in the graft. Conversely, the Dx-induced ductal cystic area was markedly reduced in the siPGC-1alpha- and Dx-treated group. CONCLUSIONS: Our results suggest that the transdifferentiation of porcine NPCCs into beta cells is influenced by the duration of the Dx treatment, which might result from the suppression of key pancreatic transcription factors. PGC-1alpha is an attractive target for modulating the deleterious effects of glucocorticoids on pancreatic stem cells.

Kim, S. W., M. Houge, et al. "Cultured human bone marrow-derived CD31(+) cells are effective for cardiac and vascular repair through enhanced angiogenic, adhesion, and anti-inflammatory effects." J Am Coll Cardiol **64**(16): 1681-94.

BACKGROUND: Cell therapy for cardiovascular disease has been limited by low engraftment of administered cells and modest therapeutic effects. Bone marrow (BM) -derived CD31(+) cells are a promising cell source owing to their high angiovasculogenic and paracrine activities. OBJECTIVES: This study sought to identify culture conditions that could augment the cell adhesion, angiogenic, and anti-inflammatory activities of BM-derived CD31(+) cells, and to determine whether these cultured CD31(+) cells are effective for cardiac and vascular repair. METHODS: CD31(+) cells were isolated from human BM by magnetic-activated cell sorting and cultured for 10 days under hematopoietic stem cell, mesenchymal stem cell, or endothelial cell culture conditions. These cells were characterized by adhesion, angiogenesis, and inflammatory assays. The best of the cultured cells were implanted into myocardial infarction (MI) and hindlimb ischemia (HLI) models to determine therapeutic effects and underlying mechanisms. RESULTS: The CD31(+) cells cultured in endothelial cell medium (EC-CD31(+) cells) showed the highest adhesion and angiogenic activities and lowest inflammatory properties in vitro compared with uncultured or other cultured CD31(+) cells. When implanted into mouse MI or HLI models, EC-CD31(+) cells improved cardiac function and repaired limb ischemia to a greater extent than uncultured CD31(+) cells. Histologically, injected EC-CD31(+) cells exhibited higher retention, neovascularization, and cardiomyocyte proliferation. Importantly, cell retention and endothelial transdifferentiation was sustained up to 1 year. CONCLUSIONS: Short-term cultured EC-CD31(+) cells have higher cell engraftment, vessel-formation, cardiomyocyte proliferation, and anti-inflammatory potential, are highly effective for both cardiac and peripheral vascular repair, and enhance survival of mice with heart failure. These cultured CD31(+) cells may be a promising source for treating ischemic cardiovascular diseases.

King, A. and P. N. Newsome "Bone marrow stem cell therapy for liver disease." Dig Dis **32**(5): 494-501.

Liver disease is a rising cause of mortality and morbidity, and treatment options remain limited. Liver transplantation is curative but limited by donor

organ availability, operative risk and long-term complications. The contribution of bone marrow (BM)-derived stem cells to tissue regeneration has been recognised and there is considerable interest in the potential benefits of BM stem cells in patients with liver disease. In chronic liver disease, deposition of fibrous scar tissue inhibits hepatocyte proliferation and leads to portal hypertension. Although initial reports had suggested transdifferentiation of stem cells into hepatocytes, the beneficial effects of BM stem cells are more likely derived from the ability to breakdown scar tissue and stimulate hepatocyte proliferation. Studies in animal models have yielded promising results, although the exact mechanisms and cell type responsible have yet to be determined. Small-scale clinical studies have quickly followed and, although primarily designed to examine safety and feasibility of this approach, have reported improvements in liver function in treated patients. Well-designed, controlled studies are required to fully determine the benefits of BM stem cell therapy.

Kong, W., M. Nuo, et al. "Kidney regeneration by non-platelet RNA-containing particle-derived cells." Clin Exp Pharmacol Physiol **40**(11): 724-34.

We found a group of non-platelet RNA-containing particles (NPRCPs) in human umbilical cord blood. These particles can aggregate, fuse and become non-nucleated cells when cocultured with nucleated cells in vitro. The non-nucleated cells further differentiate into nucleated cells expressing octamer binding transcription factor 4 (OCT4). The NPRCPs are approximately 1-5 mum in diameter, have a thin bilayer membrane, contain short RNAs and microRNAs and express OCT4, sex-determining region Y 2 (SOX2) and DEAD box polypeptide 4 (DDX4). To confirm the function of NPRCPs in vivo, we examined the effects of tail vein-injected green fluorescent protein (GFP)-labelled NPRCPs on mouse kidneys damaged by prior ischaemia and reperfusion from Day 1 to Week 6. Within 1 day of injection of NPRCPs, immunofluorescence and immunohistochemistry revealed a large number of extravasated NPRCPs in the renal calyces, damaged glomeruli and duct tubules. During the course of regeneration, NPRCPs fused into large, non-nucleated cellular structures that further became large nucleated cells to regenerate multicellular kidney tubules. In addition, many NPRCPs became tiny nucleated cellular structures that further differentiated into interstitial cells in connective tissue. The extravasated NPRCPs also arranged themselves into non-cell glomerular structures before further regenerating into nucleated cells of the glomerulus. In conclusion, the results demonstrate that, via different patterns of differentiation, NPRCP-derived cells can regenerate mouse kidney tissue damaged by ischaemia.

Lamouille, S., J. Xu, et al. "Molecular mechanisms of epithelial-mesenchymal transition." Nat Rev Mol Cell Biol **15**(3): 178-96.

The transdifferentiation of epithelial cells into motile mesenchymal cells, a process known as epithelial-mesenchymal transition (EMT), is integral in development, wound healing and stem cell behaviour, and contributes pathologically to fibrosis and cancer progression. This switch in cell differentiation and behaviour is mediated by key transcription factors, including SNAIL, zinc-finger E-box-binding (ZEB) and basic helix-loop-helix transcription factors, the functions of which are finely regulated at the transcriptional, translational and post-translational levels. The reprogramming of gene expression during EMT, as well as non-transcriptional changes, are initiated and controlled by signalling pathways that respond to extracellular cues. Among these, transforming growth factor-beta (TGFbeta) family signalling has a predominant role; however, the convergence of signalling pathways is essential for EMT.

Laos, M., T. Anttonen, et al. "DNA damage signaling regulates age-dependent proliferative capacity of quiescent inner ear supporting cells." Aging (Albany NY) **6**(6): 496-510.

Supporting cells (SCs) of the cochlear (auditory) and vestibular (balance) organs hold promise as a platform for therapeutic regeneration of the sensory hair cells. Prior data have shown proliferative restrictions of adult SCs forced to re-enter the cell cycle. By comparing juvenile and adult SCs in explant cultures, we have here studied how proliferative restrictions are linked with DNA damage signaling. Cyclin D1 overexpression, used to stimulate cell cycle re-entry, triggered higher proliferative activity of juvenile SCs. Phosphorylated form of histone H2AX (gammaH2AX) and p53 binding protein 1 (53BP1) were induced in a foci-like pattern in SCs of both ages as an indication of DNA double-strand break formation and activated DNA damage response. Compared to juvenile SCs, gammaH2AX and the repair protein Rad51 were resolved with slower kinetics in adult SCs, accompanied by increased apoptosis. Consistent with thein vitro data, in a Rb mutant mouse model in vivo, cell cycle re-entry of SCs was associated with gammaH2AX foci induction. In contrast to cell cycle reactivation, pharmacological stimulation of SC-to-hair-cell transdifferentiation in vitro did not trigger gammaH2AX. Thus, DNA damage and its prolonged

resolution are critical barriers in the efforts to stimulate proliferation of the adult inner ear SCs.

Lee, J. S., S. Y. An, et al. "Transdifferentiation of human periodontal ligament stem cells into pancreatic cell lineage." Cell Biochem Funct **32**(7): 605-11.

Human periodontal ligament-derived stem cells (PDLSCs) demonstrate self-renewal capacity and multilineage differentiation potential. In this study, we investigated the transdifferentiation potential of human PDLSCs into pancreatic islet cells. To form three-dimensional (3D) clusters, PDLSCs were cultured in Matrigel with media containing differentiation-inducing agents. We found that after 6 days in culture, PDLSCs underwent morphological changes resembling pancreatic islet-like cell clusters (ICCs). The morphological characteristics of PDLSC-derived ICCs were further assessed using scanning electron microscopy analysis. Using reverse transcription-polymerase chain reaction analysis, we found that pluripotency genes were downregulated, whereas early endoderm and pancreatic differentiation genes were upregulated, in PDLSC-derived ICCs compared with undifferentiated PDLSCs. Furthermore, we found that PDLSC-derived ICCs were capable of secreting insulin in response to high concentrations of glucose, validating their functional differentiation into islet cells. Finally, we also performed dithizone staining, as well as immunofluorescence assays and fluorescence-activated cell sorting analysis for pancreatic differentiation markers, to confirm the differentiation status of PDLSC-derived ICCs. These results demonstrate that PDLSCs can transdifferentiate into functional pancreatic islet-like cells and provide a novel, alternative cell population for pancreatic repair.

Leri, A., M. Rota, et al. "Origin of cardiomyocytes in the adult heart." Circ Res **116**(1): 150-66.

This review article discusses the mechanisms of cardiomyogenesis in the adult heart. They include the re-entry of cardiomyocytes into the cell cycle; dedifferentiation of pre-existing cardiomyocytes, which assume an immature replicating cell phenotype; transdifferentiation of hematopoietic stem cells into cardiomyocytes; and cardiomyocytes derived from activation and lineage specification of resident cardiac stem cells. The recognition of the origin of cardiomyocytes is of critical importance for the development of strategies capable of enhancing the growth response of the myocardium; in fact, cell therapy for the decompensated heart has to be based on the acquisition of this fundamental biological knowledge.

Li, W., J. Wu, et al. "Notch inhibition induces mitotically generated hair cells in mammalian cochleae via activating the Wnt pathway." Proc Natl Acad Sci U S A **112**(1): 166-71.

The activation of cochlear progenitor cells is a promising approach for hair cell (HC) regeneration and hearing recovery. The mechanisms underlying the initiation of proliferation of postnatal cochlear progenitor cells and their transdifferentiation to HCs remain to be determined. We show that Notch inhibition initiates proliferation of supporting cells (SCs) and mitotic regeneration of HCs in neonatal mouse cochlea in vivo and in vitro. Through lineage tracing, we identify that a majority of the proliferating SCs and mitotic-generated HCs induced by Notch inhibition are derived from the Wnt-responsive leucine-rich repeat-containing G protein-coupled receptor 5 (Lgr5(+)) progenitor cells. We demonstrate that Notch inhibition removes the brakes on the canonical Wnt signaling and promotes Lgr5(+) progenitor cells to mitotically generate new HCs. Our study reveals a new function of Notch signaling in limiting proliferation and regeneration potential of postnatal cochlear progenitor cells, and provides a new route to regenerate HCs from progenitor cells by interrupting the interaction between the Notch and Wnt pathways.

Liberko, M., K. Kolostova, et al. "Essentials of circulating tumor cells for clinical research and practice." Crit Rev Oncol Hematol **88**(2): 338-56.

The major cause of death due to cancer is its metastatic deposit in numerous tissues and organs. The metastatic process requires the migration of malignant cells from primary sites to distant environments. Even for tumors initially spreading through lymphatic vessels, hematogenous transport is the most common metastatic pathway. The detachment of cancer cells from a primary tumor into the blood stream is called epithelial-mesenchymal transition (EMT). As these cells circulate further in the bloodstream they are known as circulating tumor cells (CTCs). The CTC population is highly resilient, enabling the cells to colonize a foreign microenvironment. Alternatively, cancer stem cells (CSCs) may arise from differentiated cancer cells through EMT and an embryonic transdifferentiation process. The presence of CTCs/CSCs in blood seems to be a determining factor of metastasis. This paper reviews various methods of clinical cancer detection as well as the biology and molecular characterization of CTCs/CSCs. Our goal was to summarize clinical studies which used CTC/CSCs for prognosis in patients with breast, colorectal, prostate, lung, ovarian, and bladder cancer.

Lin, C. Y., J. R. Yang, et al. "Microarray analysis of gene expression of bone marrow stem cells cocultured

with salivary acinar cells." J Formos Med Assoc **112**(11): 713-20.

BACKGROUND/PURPOSE: Our previous work has demonstrated that rat bone marrow stem cells (BMSCs) can transdifferentiate into alpha-amylase-producing cells after coculture with rat submandibular gland acinar cells. These transdifferentiated cells may be used for regeneration of damaged salivary gland. The purpose of this study was to investigate the global gene expression of rat BMSCs cocultured with rat submandibular gland acinar cells and the factors inducing this transdifferentiation. METHODS: Rat BMSCs were indirectly cocultured with rat submandibular gland acinar cells by using the double chamber system for 5 and 10 days. The global gene expression of BMSCs during transdifferentiation into acinar cells was investigated by microarray analysis. RESULTS: A total of 45,018 probes were used and 41,012 genes were detected. After coculture for 5 days, 1409 genes were upregulated more than twofold and 1417 genes were downregulated more than twofold ($p<0.005$). Moreover, after coculture for 10 days, 1356 genes were upregulated more than twofold and 1231 genes were downregulated more than twofold ($p<0.005$). Bone morphogenetic protein (BMP)-6 was one of the top-ranked upregulated genes. The hub genes were interleukin-6 and CCAAT/enhancer-binding protein beta (CEBPB) in the early and late response gene groups, respectively. CONCLUSION: This is believed to be the first study on the global gene expression of rat BMSCs cocultured with rat acinar cells. Many genes related to the function of salivary acinar cells such as those responsible for the production of alpha-amylase protein were upregulated and many genes related to the differentiation of BMSCs into adipocytes and osteoblasts were downregulated. In addition, BMP-6 gene was found to be highly upregulated. We proposed that three target genes, BMP-6, interleukin-6 and CEBPB, play important roles in the transdifferentiation of BMSCs into acinar cells, and are worthy of further investigation.

Liu, C. and H. Wu "From Beta cell replacement to beta cell regeneration: implications for antidiabetic therapy." J Diabetes Sci Technol **8**(6): 1221-6.

Diabetes is affecting more than 25.8 million people in the United States, causing huge burden on the health care system and economy. Insulin injection, which is the predominant treatment for diabetes, is incapable of replenishing the lost insulin-producing beta cell in patients. Restoring beta cell mass through replacement therapy such as islet transplantation or beta cell regeneration through in vitro and in vivo strategies has attracted particular attentions in the field due to its potential to cure diabetes. In the aspect of islet transplantation, gene therapy, stem cell therapy, and more biocompatible immunosuppressive drugs have been tested in various preclinical animal models to improve the longevity and function of human islets against the posttransplantation challenges. In the islet regeneration aspect, insulin-producing cells have been generated through in vitro transdifferentiation of stem cells and other types of cells and demonstrated to be capable of glycemic control. Moreover, several biomarkers including cell-surface receptors, soluble factors, and transcriptional factors have been identified or rediscovered in mediating the process of beta cell proliferation in rodents. This review summarizes the current progress and hurdles in the preclinical efforts in resurrecting beta cells. It may provide some useful insights into the future drug discovery for antidiabetic purposes.

Liu, W. H., F. Q. Song, et al. "The multiple functional roles of mesenchymal stem cells in participating in treating liver diseases." J Cell Mol Med **19**(3): 511-20.

Mesenchymal stem cells (MSCs) are a group of stem cells derived from the mesodermal mesenchyme. MSCs can be obtained from a variety of tissues, including bone marrow, umbilical cord tissue, umbilical cord blood, peripheral blood and adipose tissue. Under certain conditions, MSCs can differentiate into many cell types both in vitro and in vivo, including hepatocytes. To date, four main strategies have been developed to induce the transdifferentiation of MSCs into hepatocytes: addition of chemical compounds and cytokines, genetic modification, adjustment of the micro-environment and alteration of the physical parameters used for culturing MSCs. Although the phenomenon of transdifferentiation of MSCs into hepatocytes has been described, the detailed mechanism is far from clear. Generally, the mechanism is a cascade reaction whereby stimulating factors activate cellular signalling pathways, which in turn promote the production of transcription factors, leading to hepatic gene expression. Because MSCs can give rise to hepatocytes, they are promising to be used as a new treatment for liver dysfunction or as a bridge to liver transplantation. Numerous studies have confirmed the therapeutic effects of MSCs on hepatic fibrosis, cirrhosis and other liver diseases, which may be related to the differentiation of MSCs into functional hepatocytes. In addition to transdifferentiation into hepatocytes, when MSCs are used to treat liver disease, they may also inhibit hepatocellular apoptosis and secrete various bioactive molecules to promote liver regeneration. In this review, the capacity and molecular mechanism of MSC transdifferentiation, and the therapeutic effects of MSCs on liver diseases are thoroughly discussed.

Lu, J., R. Jaafer, et al. "Transdifferentiation of pancreatic alpha-cells into insulin-secreting cells: From experimental models to underlying mechanisms." World J Diabetes **5**(6): 847-53.

Pancreatic insulin-secreting beta-cells are essential regulators of glucose metabolism. New strategies are currently being investigated to create insulin-producing beta cells to replace deficient beta cells, including the differentiation of either stem or progenitor cells, and the newly uncovered transdifferentiation of mature non-beta islet cell types. However, in order to correctly drive any cell to adopt a new beta-cell fate, a better understanding of the in vivo mechanisms involved in the plasticity and biology of islet cells is urgently required. Here, we review the recent studies reporting the phenomenon of transdifferentiation of alpha cells into beta cells by focusing on the major candidates and contexts revealed to be involved in adult beta-cell regeneration through this process. The possible underlying mechanisms of transdifferentiation and the interactions between several key factors involved in the process are also addressed. We propose that it is of importance to further study the molecular and cellular mechanisms underlying alpha- to beta-cell transdifferentiation, in order to make beta-cell regeneration from alpha cells a relevant and realizable strategy for developing cell-replacement therapy.

Lu, Y., H. Zhu, et al. "Knockdown of Oct4 and Nanog expression inhibits the stemness of pancreatic cancer cells." Cancer Lett **340**(1): 113-23.

Pancreatic cancer is notorious for its difficult diagnosis at early stage and poor recurrence-free prognosis. This study aimed to investigate the possible involvement of Oct4 and Nanog in pancreatic cancer. The high expressions of Oct4 and Nanog in human pancreatic cancer tissues were found to indicate a worse prognostic value of patients. The pancreatic cancer stem cells (PCSCs) that isolated from PANC-1 cell line by flow cytometry exhibited high expressions of Oct4 and Nanog. To investigate whether Oct4 and Nanog play crucial role in maintaining the stemness of PCSCs, double knockdown of Oct4 and Nanog demonstrated that Oct4 and Nanog significantly reduced proliferation, migration, invasion, chemoresistance, and tumorigenesis of PCSCs in vitro and in vivo. The altered expression of the genes related to pancreatic carcinogenesis, metastasis, drug resistance and epithelial-mesenchymal transdifferentiation (EMT) might affect the biological characteristics of PCSCs. Our results suggest that Oct4 and Nanog may serve as a potential marker of prognosis and a novel target of therapy for pancreatic cancer.

Lysy, P. A., G. C. Weir, et al. "Making beta cells from adult cells within the pancreas." Curr Diab Rep **13**(5): 695-703.

Cell therapy is currently considered as a potential therapeutic alternative to traditional treatments of diabetes. Islet and whole pancreas transplantations provided the proof-of-concept of glucose homeostasis restoration after replenishment of the deficiency of beta cells responsible for the disease. Current limitations of these procedures have led to the search for strategies targeting replication of pre-existing beta cells or transdifferentiation of progenitors and adult cells. These investigations revealed an unexpected plasticity towards beta cells of adult cells residing in pancreatic epithelium (eg, acinar, duct, and alpha cells). Here we discuss recent developments in beta-cell replication and beta-cell transdifferentiation of adult epithelial pancreatic cells, with an emphasis on techniques with a potential for clinical translation.

Marelli, B., C. E. Ghezzi, et al. "Fabrication of injectable, cellular, anisotropic collagen tissue equivalents with modular fibrillar densities." Biomaterials **37**: 183-93.

Technological improvements in collagen gel fabrication are highly desirable as they may enable significant advances in the formation of tissue-equivalent biomaterials for regenerative medicine, three-dimensional (3D) in vitro tissue models, and injectable scaffolds for cell and drug delivery applications. Thus, strategies to modulate collagen gel fibrillar density and organization in the mesostructure have been pursued to fabricate collagenous matrices with extracellular matrix-like features. Herein, we introduce a robust and simple method, namely gel aspiration-ejection (GAE), to engineer 3D, anisotropic, cell seeded, injectable dense collagen (I-DC) gels with controllable fibrillar densities, without the use of crosslinking. GAE allows for the hybridization of collagen gels with bioactive agents for increased functionality and supports highly aligned homogenous cell seeding, thus providing I-DC gels with distinct properties when compared to isotropic DC gels of random fibrillar orientation. The hybridization of I-DC with anionic fibroin derived polypeptides resulted in the nucleation of carbonated hydroxyapatite within the aligned nanofibrillar network upon exposure to simulated body fluid, yielding a 3D, anisotropic, mineralized collagen matrix. In addition, I-DC gels accelerated the osteoblastic differentiation of seeded murine mesenchymal stem cells (m-MSCs) when exposed to osteogenic supplements, which resulted in the cell-mediated, bulk mineralization of the osteoid-like gels. In addition, and upon exposure to neuronal transdifferentiation medium, I-DC gels supported and

accelerated the differentiation of m-MSCs toward neuronal cells. In conclusion, collagen GAE presents interesting opportunities in a number of fields spanning tissue engineering and regenerative medicine to drug and cell delivery.

Marro, S. and N. Yang "Transdifferentiation of mouse fibroblasts and hepatocytes to functional neurons." Methods Mol Biol **1150**: 237-46.

Nuclear reprogramming by defined transcription factors became of broad interest in 2006 with the work of Takahashi and Yamanaka (Cell 126:663-676, 2006), but the first example of cell fate reshaping via ectopic expression of transcription factor was provided back in 1987 when Davis and colleagues induced features of a muscle cell in fibroblast using the muscle transcription factor MyoD (Davis et al., Cell 51:987-1000, 1987). In 2010 our laboratory described how forced expression of the three neuronal transcription factors Ascl1, Brn2, and Myt1l rapidly converts mouse fibroblasts into neuronal cells that exhibit biochemical and electrophysiological properties of neurons. We named these cells induced neuronal cells (iN cells) (Vierbuchen et al., Nature 463:1035-1041, 2010; Vierbuchen and Wernig, Nat Biotechnol 29:892-907, 2011). Interestingly, iN cells can also be derived from defined endodermal cells such as primary hepatocytes, suggesting the existence of a more general reprogramming paradigm (Marro et al., Cell Stem Cell 9:374-382, 2011). In this chapter we describe the detailed methods used to attain the direct conversion.

Marz, M., F. Seebeck, et al. "A Pitx transcription factor controls the establishment and maintenance of the serotonergic lineage in planarians." Development **140**(22): 4499-509.

In contrast to adult vertebrates, which have limited capacities for neurogenesis, adult planarians undergo constitutive cellular turnover during homeostasis and are even able to regenerate a whole brain after decapitation. This enormous plasticity derives from pluripotent stem cells residing in the planarian body in large numbers. It is still obscure how these stem cells are programmed for differentiation into specific cell lineages and how lineage identity is maintained. Here we identify a Pitx transcription factor of crucial importance for planarian regeneration. In addition to patterning defects that are co-dependent on the LIM homeobox transcription factor gene islet1, which is expressed with pitx at anterior and posterior regeneration poles, RNAi against pitx results in islet1-independent specific loss of serotonergic (SN) neurons during regeneration. Besides its expression in terminally differentiated SN neurons we found pitx in stem cell progeny committed to the SN fate. Also, intact pitx RNAi animals gradually lose SN markers, a phenotype that depends neither on increased apoptosis nor on stem cell-based turnover or transdifferentiation into other neurons. We propose that pitx is a terminal selector gene for SN neurons in planarians that controls not only their maturation but also their identity by regulating the expression of the Serotonin production and transport machinery. Finally, we made use of this function of pitx and compared the transcriptomes of regenerating planarians with and without functional SN neurons, identifying at least three new neuronal targets of Pitx.

Meseure, D., K. Drak Alsibai, et al. "Pivotal role of pervasive neoplastic and stromal cells reprogramming in circulating tumor cells dissemination and metastatic colonization." Cancer Microenviron **7**(3): 95-115.

Reciprocal interactions between neoplastic cells and their microenvironment are crucial events in carcinogenesis and tumor progression. Pervasive stromal reprogramming and remodeling that transform a normal to a tumorigenic microenvironment modify numerous stromal cells functions, status redox, oxidative stress, pH, ECM stiffness and energy metabolism. These environmental factors allow selection of more aggressive cancer cells that develop important adaptive strategies. Subpopulations of cancer cells acquire new properties associating plasticity, stem-like phenotype, unfolded protein response, metabolic reprogramming and autophagy, production of exosomes, survival to anoikis, invasion, immunosuppression and therapeutic resistance. Moreover, by inducing vascular transdifferentiation of cancer cells and recruiting endothelial cells and pericytes, the tumorigenic microenvironment induces development of tumor-associated vessels that allow invasive cells to gain access to the tumor vessels and to intravasate. Circulating cancer cells can survive in the blood stream by interacting with the intravascular microenvironment, extravasate through the microvasculature and interact with the metastatic microenvironment of target organs. In this review, we will focus on many recent paradigms involved in the field of tumor progression.

Miao, C. G., Y. Y. Yang, et al. "Wnt signaling in liver fibrosis: progress, challenges and potential directions." Biochimie **95**(12): 2326-35.

Liver fibrosis is a common wound-healing response to chronic liver injuries, including alcoholic or drug toxicity, persistent viral infection, and genetic factors. Myofibroblastic transdifferentiation (MTD) is the pivotal event during liver fibrogenesis, and research in the past few years has identified key mediators and molecular mechanisms responsible for MTD of hepatic stellate cells (HSCs). HSCs are

undifferentiated cells which play an important role in liver regeneration. Recent evidence demonstrates that HSCs derive from mesoderm and at least in part via septum transversum and mesothelium, and HSCs express markers for different cell types which derive from multipotent mesenchymal progenitors. There is a regulatory commonality between differentiation of adipocytes and that of HSC, and the shift from adipogenic to myogenic or neuronal phenotype characterizes HSC MTD. Central of this shift is a loss of expression of the master adipogenic regulator peroxisome proliferator activated receptor gamma (PPARgamma). Restored expression of PPARgamma and/or other adipogenic transcription genes can reverse myofibroblastic HSCs to differentiated cells. Vertebrate Wnt and Drosophila wingless are homologous genes, and their translated proteins have been shown to participate in the regulation of cell proliferation, cell polarity, cell differentiation, and other biological roles. More recently, Wnt signaling is implicated in human fibrosing diseases, such as pulmonary fibrosis, renal fibrosis, and liver fibrosis. Blocking the canonical Wnt signal pathway with the co-receptor antagonist Dickkopf-1 (DKK1) abrogates these epigenetic repressions and restores the gene PPARgamma expression and HSC differentiation. The identified morphogen mediated epigenetic regulation of PPARgamma and HSC differentiation also serves as novel therapeutic targets for liver fibrosis and liver regeneration. In conclusion, the Wnt signaling promotes liver fibrosis by enhancing HSC activation and survival, and we herein discuss what we currently know and what we expect will come in this field in the next future.

Mirakhori, F., B. Zeynali, et al. "Induced neural lineage cells as repair kits: so close, yet so far away." J Cell Physiol **229**(6): 728-42.

Transdifferentiation or direct reprogramming of somatic cells into neural lineage cells has provided an invaluable new tool to advance the regenerative neural medicine. Here, we provide an overview of the various strategies currently available for producing of induced neural lineage cells in vitro as well as the direct reprogramming of neural cells in vivo. We also discussing some of the challenges faced in harnessing the potential of induced neural lineage cells for biomedical applications.

Miyake, A. and M. Araki "Retinal stem/progenitor cells in the ciliary marginal zone complete retinal regeneration: a study of retinal regeneration in a novel animal model." Dev Neurobiol **74**(7): 739-56.

Our research group has extensively studied retinal regeneration in adult Xenopus laevis. However, X. laevis does not represent a suitable model for multigenerational genetics and genomic approaches. Instead, Xenopus tropicalis is considered as the ideal model for these studies, although little is known about retinal regeneration in X. tropicalis. In the present study, we showed that a complete retina regenerates at approximately 30 days after whole retinal removal. The regenerating retina was derived from the stem/progenitor cells in the ciliary marginal zone (CMZ), indicating a novel mode of vertebrate retinal regeneration, which has not been previously reported. In a previous study, we showed that in X. laevis, retinal regeneration occurs primarily through the transdifferentiation of retinal pigmented epithelial (RPE) cells. RPE cells migrate to the retinal vascular membrane and reform a new epithelium, which then differentiates into the retina. In X. tropicalis, RPE cells also migrated to the vascular membrane, but transdifferentiation was not evident. Using two tissue culture models of RPE tissues, it was shown that in X. laevis RPE culture neuronal differentiation and reconstruction of the retinal three-dimensional (3-D) structure were clearly observed, while in X. tropicalis RPE culture neither ssIII tubulin-positive cells nor 3-D retinal structure were seen. These results indicate that the two Xenopus species are excellent models to clarify the cellular and molecular mechanisms of retinal regeneration, as these animals have contrasting modes of regeneration; one mode primarily involves RPE cells and the other mode involves stem/progenitor cells in the CMZ.

Moignard, V., S. Woodhouse, et al. "Transcriptional hierarchies regulating early blood cell development." Blood Cells Mol Dis **51**(4): 239-47.

Hematopoiesis represents one of the paradigmatic systems for studying stem cell biology, but our understanding of how the hematopoietic system develops during embryogenesis is still incomplete. While many lessons have been learned from studying the mouse embryo, embryonic stem cells have come to the fore as an alternative and more tractable model to recapitulate hematopoietic development. Here we review what is known about the embryonic origin of blood from these complementary systems and how transcription factor networks regulate the emergence of hematopoietic tissue from the mesoderm. Furthermore, we have performed an integrated analysis of genome-wide microarray and ChIP-seq data sets from mouse embryos and embryonic stem (ES) cell lines deficient in key regulators and demonstrate how this type of analysis can be used to reconstruct regulatory hierarchies that both confirm existing regulatory linkages and suggest additional interactions.

Mosaad, Y. M. "Hematopoietic stem cells: an overview." Transfus Apher Sci **51**(3): 68-82.

Considerable efforts have been made in recent years in understanding the mechanisms that govern hematopoietic stem cell (HSC) origin, development, differentiation, self-renewal, aging, trafficking, plasticity and transdifferentiation. Hematopoiesis occurs in sequential waves in distinct anatomical locations during development and these shifts in location are accompanied by changes in the functional status of the stem cells and reflect the changing needs of the developing organism. HSCs make a choice of either self-renewal or committing to differentiation. The balance between self-renewal and differentiation is considered to be critical to the maintenance of stem cell numbers. It is still under debate if HSC can rejuvenate infinitely or if they do not possess "true" self-renewal and undergo replicative senescence such as any other somatic cell. Gene therapy applications that target HSCs offer a great potential for the treatment of hematologic and immunologic diseases. However, the clinical success has been limited by many factors. This review is intended to summarize the recent advances made in the human HSC field, and will review the hematopoietic stem cell from definition through development to clinical applications.

Muir, K. R., M. J. Lima, et al. "Cell therapy for type 1 diabetes." Qjm **107**(4): 253-9.

Cell therapy in the form of human islet transplantation has been a successful form of treatment for patients with type 1 diabetes for over 10 years, but is significantly limited by lack of suitable donor material. A replenishable supply of insulin-producing cells has the potential to address this problem; however to date success has been limited to a few preclinical studies. Two of the most promising strategies include differentiation of embryonic stem cells and induced pluripotent stem cells towards insulin-producing cells and transdifferentiation of acinar or other closely related cell types towards beta-cells. Here, we discuss recent progress and challenges that need to be overcome in taking cell therapy to the clinic.

Nakayama, K. H., L. Hou, et al. "Role of extracellular matrix signaling cues in modulating cell fate commitment for cardiovascular tissue engineering." Adv Healthc Mater **3**(5): 628-41.

It is generally agreed that engineered cardiovascular tissues require cellular interactions with the local milieu. Within the microenvironment, the extracellular matrix (ECM) is an important support structure that provides dynamic signaling cues in part through its chemical, physical, and mechanical properties. In response to ECM factors, cells activate biochemical and mechanotransduction pathways that modulate their survival, growth, migration, differentiation, and function. This Review describes the role of ECM chemical composition, spatial patterning, and mechanical stimulation in the specification of cardiovascular lineages, with a focus on stem cell differentiation, direct transdifferentiation, and endothelial-to-mesenchymal transition. The translational application of ECMs is discussed in the context of cardiovascular tissue engineering and regenerative medicine.

Novak, D., K. Weina, et al. "From skin to other cell types of the body." J Dtsch Dermatol Ges **12**(9): 789-92.

Regenerative medicine allows for the customization of tissues and organs which may bring hope to patients with incurable diseases and severe injuries. Therefore, reliable and safe methods for the generation of specific cell types must be established. Recently, different strategies have emerged to convert somatic cells into differentiated cells of interest. One of these strategies is cellular reprogramming, which converts somatic cells into induced pluripotent stem cells (iPSCs). These iPSCs are embryonic stem cell-like cells with almost unlimited developmental potential and can be differentiated into specific lineages. Alternatively, the method of transdifferentiation can be used to directly convert one terminally differentiated cell into another cell type. Both of these methods have proven to have the potential to push the field of cell replacement therapy forward. In this context, the skin is of particular interest because it represents an ideal source of somatic cells for reprogramming to pluripotency as well as transdifferentiation. In this review, we briefly compare both above-mentioned strategies and summarize the latest advances in this highly dynamic field of research.

Ostrakhovitch, E. A., S. Akakura, et al. "Dedifferentiation of cancer cells following recovery from a potentially lethal damage is mediated by H2S-Nampt." Exp Cell Res **330**(1): 135-50.

Recently, we reported that cancer cells that recover from a potentially lethal damage gain new phenotypic features comprised of mitochondrial structural remodeling associated with increased glycolytic dependency and drug resistance. Here, we demonstrate that a subset of cancer cells, upon recovery from a potentially lethal damage, undergo dedifferentiation and express genes, which are characteristic of undifferentiated stem cells. While these cells are competent in maintaining differentiated progeny of tumor, they also exhibit transdifferentiation

potential. Dedifferentiation is characterized by accumulation of hydrogen sulfide (H2S), which triggers up-regulation of nicotinamide phosphoribosyltransferase (Nampt) accompanied by changes in the redox state. The molecular events triggered by Nampt include elevated production of NAD(+) and up-regulation of H2S producing enzymes, cystathionine beta synthase (CBS) and cystathionase (CTH) with 3-mercaptopyruvate sulfurtransferase (MST) being detectable only in 3D spheroids. Suppression of Nampt, or inactivation of H2S producing enzymes, all reduce H2S production and reverse the ability of cells to dedifferentiate. Moreover, H2S induced stem cell markers in parental cancer cells in a manner similar to that observed in damage recovered cells. These data suggest of existence of a positive feedback loop between H2S and Nampt that controls dedifferentiation in cancer cells that recover from a potentially lethal damage.

Park, G. H., H. Jeong, et al. "Novel TAZ modulators enhance myogenic differentiation and muscle regeneration." Br J Pharmacol **171**(17): 4051-61.

BACKGROUND AND PURPOSE: The transcriptional co-activator with PDZ-binding motif (TAZ) is a key controller of mesenchymal stem cell differentiation through its nuclear localization and subsequent interaction with master transcription factors. In particular, TAZ directly associates with myoblast determining protein D (MyoD) and activates MyoD-induced myogenic gene expression, thereby enhancing myogenic differentiation. Here, we have synthesized and characterized low MW compounds modulating myogenic differentiation via induction of TAZ nuclear localization. EXPERIMENTAL APPROACH: COS7 cells stably transfected with GFP-TAZ were used in a high content imaging screen for compounds specifically enhancing nuclear localization of TAZ. We then studied the effects of such TAZ modulators on myocyte differentiation of C2C12 cells and myogenic transdifferentiation of mouse embryonic fibroblast cells in vitro and muscle regeneration in vivo. KEY RESULTS: We identified two TAZ modulators, TM-53, and its structural isomer, TM-54. Each compound strongly enhanced nuclear localization of TAZ by reducing S89-phosphorylation and dose-dependently augmented myogenic differentiation and MyoD-mediated myogenic transdifferentiation through an activation of MyoD-TAZ interaction. The myogenic stimulatory effects of TM-53 and TM-54 were impaired in the absence of TAZ, but retrieved by the restoration of TAZ. In addition, administration of TM-53 and TM-54 enhanced injury-induced muscle regeneration in vivo and attenuated myofiber injury in vitro. CONCLUSIONS AND IMPLICATIONS: The novel TAZ modulators TM-53 and TM-54 accelerated myogenic differentiation and improved muscle regeneration and function after injury, demonstrating that low MW compounds targeting the nuclear localization of TAZ have beneficial effects in skeletal muscle regeneration and in recovery from muscle degenerative diseases.

Pinzariu, A., A. Sindilar, et al. "Nutritional factors in transdifferentiation of scheletal muscles to adipocytes." Rev Med Chir Soc Med Nat Iasi **118**(3): 699-705.

A current area of interest is the determination of factors able to promote the transition from muscle to adipose tissue. The current review has highlighted that treatment of myoblasts with fatty acids (especially oleic acid) and thiazolidindiones causes conversion to adipocytes. The molecular mechanisms mediating the adipogenic action of thiazolidinediones and fatty acids in myoblasts could involve peroxisome proliferators-activated receptor-gamma (PPARgamma and CCAAT-enhancer-binding protein C/EBP. The role of 1,25-D3 in adipogenesis is mediated at the molecular level through VDR-dependent inhibition of C/EBP and PPARgamma expression and a decrease in PPARgamma transactivation activity. Vitamin D supplementation increases muscle strength and ultimately reduces the incidence of falls. Additional research is needed to fully clarify the role of nutritional factors in adipogenesis.

Powell, D. J., D. C. McFarland, et al. "The effect of nutritional status and muscle fiber type on myogenic satellite cell fate and apoptosis." Poult Sci **93**(1): 163-73.

Satellite cells (SC) are multipotential stem cells that can be induced by nutrition to alter their cellular developmental fate, which may vary depending on their fiber type origin. The objective of the current study was to determine the effect of restricting protein synthesis on inducing adipogenic transdifferentiation and apoptosis of SC originating from fibers of the fast glycolytic pectoralis major (p. major) and fast oxidative and glycolytic biceps femoris (b. femoris) muscles of the chicken. The availability of the essential sulfur amino acids Met and Cys was restricted to regulate protein synthesis during SC proliferation and differentiation. The SC were cultured and treated with 1 of 6 Met/Cys concentrations: 60/192, 30/96 (control), 7.5/24, 3/9.6, 1/3.2, or 0/0 mg/L. Reductions in Met/Cys concentrations from the control level resulted in increased lipid staining and expression of the adipogenic marker genes peroxisome proliferator-activated receptor gamma and stearoyl-CoA desaturase during differentiation in the p. major SC. Although b. femoris SC had increased lipid

staining at lower Met/Cys concentrations, there was no increase in expression of either adipogenic gene. For both muscle types, SC Met/Cys, concentration above the control increased the expression of peroxisome proliferator-activated receptor gamma and stearoyl-CoA desaturase during differentiation. As Met/Cys concentration was decreased during proliferation, a dose-dependent decline in all apoptotic cells occurred except for early apoptotic cells in the p. major, which had no treatment effect ($P < 0.05$). During differentiation, decreasing Met/Cys concentration caused an increase in early apoptotic cells in both fiber types and no effect on late apoptotic cells except for an increase in the p. major 7.5/24 mg/L of Met/Cys treatment. In general, the viability of the SC was unaffected by the Met/Cys concentration except during proliferation in the p. major 0/0 mg/L of Met/Cys treatment, which increased SC viability. These data demonstrate the effect of nutrition on SC transdifferentiation to an adipogenic lineage and apoptosis, and the effect of fiber type on this response in an in vitro context.

Price, J. D., K. Y. Park, et al. "The Ink4a/Arf locus is a barrier to direct neuronal transdifferentiation." J Neurosci **34**(37): 12560-7.

Non-neurogenic cell types, such as cortical astroglia and fibroblasts, can be directly converted into neurons by the overexpression of defined transcription factors. Normally, the cellular phenotype of such differentiated cells is remarkably stable and resists direct cell transdifferentiation. Here we show that the Ink4a/Arf (also known as Cdkn2a) locus is a developmental barrier to direct neuronal transdifferentiation induced by transcription factor overexpression. With serial passage in vitro, wild-type postnatal cortical astroglia become progressively resistant to Dlx2-induced neuronal transdifferentiation. In contrast, the neurogenic competence of Ink4a/Arf-deficient astroglia is both greatly increased and does not diminish through serial cell culture passage. Electrophysiological analysis further demonstrates the neuronal identity of cells induced from Ink4a/Arf-null astroglia, and short hairpin RNA-mediated acute knockdown of p16Ink4a and p19Arf p16(Ink4a) and p19(Arf) indicates that these gene products function postnatally as a barrier to cellular transdifferentiation. Finally, we found that mouse fibroblasts deficient for Ink4a/Arf also exhibit greatly enhanced transcription factor-induced neuronal induction. These data indicate that Ink4a/Arf is a potent barrier to direct neuronal transdifferentiation and further suggest that this locus functions normally in the progressive developmental restriction of postnatal astrocytes.

Prokesch, A., A. Smorlesi, et al. "Molecular aspects of adipoepithelial transdifferentiation in mouse mammary gland." Stem Cells **32**(10): 2756-66.

The circular, reversible conversion of the mammary gland during pregnancy and involution is a paradigm of physiological tissue plasticity. The two most prominent cell types in mammary gland, adipocytes and epithelial cells, interact in an orchestrated way to coordinate this process. Previously, we showed that this conversion is at least partly achieved by reciprocal transdifferentiation between mammary adipocytes and lobulo-alveolar epithelial cells. Here, we aim to shed more light on the regulators of mammary transdifferentiation. Using immunohistochemistry with cell type-specific lipid droplet-coating markers (Perilipin1 and 2), we show that cells with an intermediate adipoepithelial phenotype exist during and after pregnancy. Nuclei of cells with similar transitional structural characteristics are highly positive for Elf5, a master regulator of alveologenesis. In cultured adipocytes, we could show that transient and stable ectopic expression of Elf5 induces expression of the milk component whey acidic protein, although the general adipocyte phenotype is not affected suggesting that additional pioneering factors are necessary. Furthermore, the lack of transdifferentiation of adipocytes during pregnancy after clearing of the epithelial compartment indicates that transdifferentiation signals must emanate from the epithelial part. To explore candidate genes potentially involved in the transdifferentiation process, we devised a high-throughput gene expression study to compare cleared mammary fat pads with developing, contralateral controls at several time points during pregnancy. Incorporation of bioinformatic predictions of secretory proteins provides new insights into possible paracrine signaling pathways and downstream transdifferentiation factors. We discuss a potential role for osteopontin (secreted phosphoprotein 1 [Spp1]) signaling through integrins to induce adipoepithelial transdifferentiation.

Rodriguez-Ubreva, J., L. Ciudad, et al. "C/EBPa-mediated activation of microRNAs 34a and 223 inhibits Lef1 expression to achieve efficient reprogramming into macrophages." Mol Cell Biol **34**(6): 1145-57.

MicroRNAs (miRNAs) exert negative effects on gene expression and influence cell lineage choice during hematopoiesis. C/EBPa-induced pre-B cell-to-macrophage transdifferentiation provides an excellent model to investigate the contribution of miRNAs to hematopoietic cell identity, especially because the two cell types involved fall into separate lymphoid and myeloid branches. In this process, efficient repression of the B cell-specific program is essential to ensure

transdifferentation and macrophage function. miRNA profiling revealed that upregulation of miRNAs is highly predominant compared with downregulation and that C/EBPa directly regulates several upregulated miRNAs. We also determined that miRNA 34a (miR-34a) and miR-223 sharply accelerate C/EBPa-mediated transdifferentiation, whereas their depletion delays this process. These two miRNAs affect the transdifferentiation efficiency and activity of macrophages, including their lipopolysaccharide (LPS)-dependent inflammatory response. miR-34a and miR-223 directly target and downregulate the lymphoid transcription factor Lef1, whose ectopic expression delays transdifferentiation to an extent similar to that seen with miR-34a and miR-223 depletion. In addition, ectopic introduction of Lef1 in macrophages causes upregulation of B cell markers, including CD19, Pax5, and Ikzf3. Our report demonstrates the importance of these miRNAs in ensuring the erasure of key B cell transcription factors, such as Lef1, and reinforces the notion of their essential role in fine-tuning the control required for establishing cell identity.

Ruggieri, M., G. Riboldi, et al. "Induced neural stem cells: methods of reprogramming and potential therapeutic applications." Prog Neurobiol **114**: 15-24.

Developmental studies and experimental data have enabled us to assert that the terminal cell differentiation state is reversible, and that altering the balance of specific transcription factors could be a powerful strategy for inducing pluripotency. Due to the risks related to using induced pluripotent cells in clinical applications, biologists are now striving to develop methods to induce a committed differentiated cell type by direct conversion of another cell line. Several reprogramming factors have been discovered, and some cellular phenotypes have been obtained by novel transdifferentiation processes. It has been recently demonstrated that induced neural stem cells (iNSCs) can be obtained from rodent and human somatic cells, like fibroblasts, through the forced expression of defined transcription factors. To date, two different approaches have been successfully used to obtain iNSCs: a direct method and an indirect method that involves an intermediate destabilized state. The possibility to induce characterized iNSCs from human cells, e.g. fibroblasts, has opened new horizons for research in human disease modelling and cellular therapeutic applications in the neurological field. This review focuses on reported reprogramming techniques and innovative techniques that can be further explored in this area, as well as on the criteria for the phenotypic characterization of iNSCs and their use in developing novel therapeutic strategies for neurological diseases.

Sayed, N., W. T. Wong, et al. "Transdifferentiation of human fibroblasts to endothelial cells: role of innate immunity." Circulation **131**(3): 300-9.

BACKGROUND: Cell fate is fluid and may be altered experimentally by the forced expression of master regulators mediating cell lineage. Such reprogramming has been achieved with the use of viral vectors encoding transcription factors. We recently discovered that the viral vectors are more than passive vehicles for transcription factors because they participate actively in the process of nuclear reprogramming to pluripotency by increasing epigenetic plasticity. On the basis of this recognition, we hypothesized that small-molecule activators of toll-like receptor 3, together with external microenvironmental cues that drive endothelial cell (EC) specification, might be sufficient to induce transdifferentiation of fibroblasts into ECs (induced ECs). METHODS AND RESULTS: We show that toll-like receptor 3 agonist Poly I:C, combined with exogenous EC growth factors, transdifferentiated human fibroblasts into ECs. These induced ECs were comparable to human dermal microvascular ECs in immunohistochemical, genetic, and functional assays, including the ability to form capillary-like structures and to incorporate acetylated low-density lipoprotein. Furthermore, induced ECs significantly improved limb perfusion and neovascularization in the murine ischemic hindlimb. Finally, using genetic knockdown studies, we found that the effective transdifferentiation of human fibroblasts to ECs requires innate immune activation. CONCLUSIONS: This study suggests that manipulation of innate immune signaling may be generally used to modify cell fate. Because similar signaling pathways are activated by damage-associated molecular patterns, epigenetic plasticity induced by innate immunity may play a fundamental role in transdifferentiation during wound healing and regeneration. Finally, this study is a first step toward development of a small-molecule strategy for therapeutic transdifferentiation for vascular disease.

Shalaby, R. H., L. A. Rashed, et al. "Hematopoietic stem cells derived from human umbilical cord ameliorate cisplatin-induced acute renal failure in rats." Am J Stem Cells **3**(2): 83-96.

Injury to a target organ can be sensed by bone marrow stem cells that migrate to the site of damage, undergo differentiation, and promote structural and functional repair. This remarkable stem cell capacity prompted an investigation of the potential of mesenchymal and hematopoietic stem cells to cure acute renal failure. On the basis of the recent demonstration that hematopoietic stem cells (HSCs) can differentiate into renal cells, the current study

tested the hypothesis that HSCs can contribute to the regeneration of renal tubular epithelial cells after renal injury. HSCs from human umbilical cord blood which isolated and purified by magnetic activated cell sorting were transplanted intraperitoneal into acute renal failure (ARF) rats which was established by a single dose of cisplatin 5 mg/kg for five days. The Study was carried on 48 male white albino rats, of average weight 120-150 gm. The animals were divided into 4 groups, Group one Served as control and received normal saline throughout the experiments. Group two (model control) received a single dose of cisplatin. Group three and four male-albino rats with induced ARF received interapritoneally (HSCs) at two week and four week respectively. Injection of a single dose of cisplatin resulted in a significant increase in serum creatinine and urea levels, histo-pathological examination of kidney tissue from cisplatin showed severe nephrotoxicity in which 50-75% of glomeruli and renal tubules exhibited massive degenerative change. Four weeks after HSC transplantation, Serum creatinine and urea nitrogen decreased 3.5 times and 2.1 times as well as HGF, IGF-1, VEGF and P53 using quantitative real-time PCR increased 4.3 times, 3.2, 2.4 and 4.2 times compared to ARF groups, respectively. The proliferation of cell nuclear antigen (PCNA)-positive cells (500.083+/-35.167) was higher than that in the cisplatin groups (58.612+/-15.743). In addition, the transplanted umbilical cord hematopoietic stem cells UC-HSCs could reside in local injury sites, leading to the relief of hyperemia and inflammation, but no obvious transdifferentiation into renal-like cells. The results lay the foundation for further study on the potential application of UC-HSCs in human disease and Because of their availability; HSC may be useful for cell replacement therapy of acute renal failure.

Shoshani, O. and D. Zipori "Stress as a fundamental theme in cell plasticity." Biochim Biophys Acta **1849**(4): 371-377.

Over a decade of intensive investigation of the possible plasticity of mammalian cells has eventually substantiated that mammalian species are endowed with a remarkable capacity to change mature cell fates. We review below the evidence for the occurrence of processes such as dedifferentiation and transdifferentiation within mammalian tissues in vivo, and in cells removed from their protective microenvironment and seeded in culture under conditions poorly resembling their physiological state in situ. Overall, these studies point to one major conclusion: stressful conditions, whether due to in vivo tissue damage or otherwise to isolation of cells from their in vivo restrictive niches, lead to extreme fate changes. Some examples of dedifferentiation are discussed in detail showing that rare cells within the population tend to turn back into less mature ones due to severe cell damage. It is proposed that cell stress, mechanistically sensed by isolation from neighboring cells, leads to dedifferentiation, in an attempt to build a new stem cell reservoir for subsequent regeneration of the damaged tissue. This article is part of a Special Issue entitled: Stress as a fundamental theme in cell plasticity.

Shu, J., K. Zhang, et al. "GATA family members as inducers for cellular reprogramming to pluripotency." Cell Res **25**(2): 169-80.

Members of the GATA protein family play important roles in lineage specification and transdifferentiation. Previous reports show that some members of the GATA protein family can also induce pluripotency in somatic cells by substituting for Oct4, a key pluripotency-associated factor. However, the mechanism linking lineage-specifying cues and the activation of pluripotency remains elusive. Here, we report that all GATA family members can substitute for Oct4 to induce pluripotency. We found that all members of the GATA family could inhibit the overrepresented ectodermal-lineage genes, which is consistent with previous reports indicating that a balance of different lineage-specifying forces is important for the restoration of pluripotency. A conserved zinc-finger DNA-binding domain in the C-terminus is critical for the GATA family to induce pluripotency. Using RNA-seq and ChIP-seq, we determined that the pluripotency-related gene Sall4 is a direct target of GATA family members during reprogramming and serves as a bridge linking the lineage-specifying GATA family to the pluripotency circuit. Thus, the GATA family is the first protein family of which all members can function as inducers of the reprogramming process and can substitute for Oct4. Our results suggest that the role of GATA family in reprogramming has been underestimated and that the GATA family may serve as an important mediator of cell fate conversion.

Solaimani Kartalaei, P., T. Yamada-Inagawa, et al. "Whole-transcriptome analysis of endothelial to hematopoietic stem cell transition reveals a requirement for Gpr56 in HSC generation." J Exp Med **212**(1): 93-106.

Hematopoietic stem cells (HSCs) are generated via a natural transdifferentiation process known as endothelial to hematopoietic cell transition (EHT). Because of small numbers of embryonal arterial cells undergoing EHT and the paucity of markers to enrich for hemogenic endothelial cells (ECs [HECs]), the genetic program driving HSC emergence is largely unknown. Here, we use a highly sensitive

RNAseq method to examine the whole transcriptome of small numbers of enriched aortic HSCs, HECs, and ECs. Gpr56, a G-coupled protein receptor, is one of the most highly up-regulated of the 530 differentially expressed genes. Also, highly up-regulated are hematopoietic transcription factors, including the "heptad" complex of factors. We show that Gpr56 (mouse and human) is a target of the heptad complex and is required for hematopoietic cluster formation during EHT. Our results identify the processes and regulators involved in EHT and reveal the surprising requirement for Gpr56 in generating the first HSCs.

Sondag, G. R., S. Salihoglu, et al. "Osteoactivin induces transdifferentiation of C2C12 myoblasts into osteoblasts." J Cell Physiol **229**(7): 955-66.

Osteoactivin (OA) is a novel osteogenic factor important for osteoblast differentiation and function. Previous studies showed that OA stimulates matrix mineralization and transcription of osteoblast specific genes required for differentiation. OA plays a role in wound healing and its expression was shown to increase in post fracture calluses. OA expression was reported in muscle as OA is upregulated in cases of denervation and unloading stress. The regulatory mechanisms of OA in muscle and bone have not yet been determined. In this study, we examined whether OA plays a role in transdifferentiation of C2C12 myoblast into osteoblasts. Infected C2C12 with a retroviral vector overexpressing OA under the CMV promoter were able to transdifferentiate from myoblasts into osteoblasts. Immunofluorescence analysis showed that skeletal muscle marker MF-20 was severely downregulated in cells overexpressing OA and contained significantly less myotubes compared to uninfected control. C2C12 myoblasts overexpressing OA showed an increase in expression of bone specific markers such as alkaline phosphatase and alizarin red staining, and also showed an increase in Runx2 protein expression. We also detected increased levels of phosphorylated focal adhesion kinase (FAK) in C2C12 myoblasts overexpressing OA compared to control. Taken together, our results suggest that OA is able to induce transdifferentiation of myoblasts into osteoblasts through increasing levels of phosphorylated FAK.

Sun, L. N., X. L. Jiang, et al. "Transdifferentiation of differentiated ovary into functional testis by long-term treatment of aromatase inhibitor in Nile tilapia." Endocrinology **155**(4): 1476-88.

Females with differentiated ovary of a gonochoristic fish, Nile tilapia, were masculinized by long-term treatment with an aromatase inhibitor (Fadrozole) in the present study. The reversed gonads developed into functional testes with fertile sperm. The longer the fish experienced sex differentiation, the longer treatment time was needed for successful sex reversal. Furthermore, Fadrozole-induced sex reversal, designated as secondary sex reversal (SSR), was successfully rescued by supplement of exogenous 17beta-estradiol. Gonadal histology, immunohistochemistry, transcriptome, and serum steroid level were analyzed during SSR. The results indicated that spermatogonia were transformed from oogonia or germline stem cell-like cells distributed in germinal epithelium, whereas Leydig and Sertoli cells probably came from the interstitial cells and granulosa cells of the ovarian tissue, respectively. The transdifferentiation of somatic cells, as indicated by the appearance of doublesex- and Mab-3-related transcription factor 1 (pre-Sertoli cells) and cytochrome P450, family 11, subfamily B, polypeptide 2 (pre-Leydig cells)-positive cells in the ovary, provided microniche for the transdifferentiation of germ cells. Decrease of serum 17beta-estradiol was detected earlier than increase of serum 11-ketotestosterone, indicating that decrease of estrogen was the cause, whereas increase of androgen was the consequence of SSR. The sex-reversed gonad displayed more similarity in morphology and histology with a testis, whereas the global gene expression profiles remained closer to the female control. Detailed analysis indicated that transdifferentiation was driven by suppression of female pathway genes and activation of male pathway genes. In short, SSR provides a good model for study of sex reversal in teleosts and for understanding of sex determination and differentiation in nonmammalian vertebrates.

Swart, J. F. and N. M. Wulffraat "Mesenchymal stromal cells for treatment of arthritis." Best Pract Res Clin Rheumatol **28**(4): 589-603.

Patients with refractory inflammatory arthritis can still respond favourable to autologous haematopoietic stem cell transplantation. However, this treatment has a high morbidity and even 5% mortality. Mesenchymal stromal cells (MSC), a subset of the non-haematopoietic stromal cells obtained from bone marrow, were found to have a strong immunosuppressive effect. MSC treatment is explored in many diseases like diabetes, SLE, MS and RA. This review covers all relevant literature regarding MSC treatment of inflammatory arthritis (RA and JIA). This review contains data of in vitro studies, animal studies and clinical studies. The following subjects will be discussed in detail: properties of MSC, presence of MSC in the joint, intra-articular versus intravenous route, autologous versus allogeneic, ideal source of MSC, distribution, transdifferentiation, engraftment, rejection, efficacy and toxicology. After reading this

review the reader will be totally updated in this quickly evolving field of MSC therapy.

Tetteh, P. W., H. F. Farin, et al. "Plasticity within stem cell hierarchies in mammalian epithelia." Trends Cell Biol **25**(2): 100-8.

Tissue homeostasis and regeneration are fueled by resident stem cells that have the capacity to self-renew, and to generate all the differentiated cell types that characterize a particular tissue. Classical models of such cellular hierarchies propose that commitment and differentiation occur unidirectionally, with the arrows 'pointing away' from the stem cell. Recent studies, all based on genetic lineage tracing, describe various strategies employed by epithelial stem cell hierarchies to replace damaged or lost cells. While transdifferentiation from one tissue type into another ('metaplasia') appears to be generally forbidden in nonpathological contexts, plasticity within an individual tissue stem cell hierarchy may be much more common than previously appreciated. In this review, we discuss recent examples of such plasticity in selected mammalian epithelia, highlighting the different modes of regeneration and their implications for our understanding of cellular hierarchy and tissue self-renewal.

Thoma, E. C., C. Merkl, et al. "Chemical conversion of human fibroblasts into functional Schwann cells." Stem Cell Reports **3**(4): 539-47.

Direct transdifferentiation of somatic cells is a promising approach to obtain patient-specific cells for numerous applications. However, conversion across germ-layer borders often requires ectopic gene expression with unpredictable side effects. Here, we present a gene-free approach that allows efficient conversion of human fibroblasts via a transient progenitor stage into Schwann cells, the major glial cell type of peripheral nerves. Using a multikinase inhibitor, we transdifferentiated fibroblasts into transient neural precursors that were subsequently further differentiated into Schwann cells. The resulting induced Schwann cells (iSCs) expressed numerous Schwann cell-specific proteins and displayed neurosupportive and myelination capacity in vitro. Thus, we established a strategy to obtain mature Schwann cells from human postnatal fibroblasts under chemically defined conditions without the introduction of ectopic genes.

Tsai, H. L., W. T. Chiu, et al. "Different forms of tenascin-C with tenascin-R regulate neural differentiation in bone marrow-derived human mesenchymal stem cells." Tissue Eng Part A **20**(13-14): 1908-21.

Mesenchymal stem cells (MSCs) are currently thought to transdifferentiate into neural lineages under specific microenvironments. Studies have reported that the tenascin family members, tenascin-C (TnC) and tenascin-R (TnR), regulate differentiation and migration, in addition to neurite outgrowth and survival in numerous types of neurons and mesenchymal progenitor cells. However, the mechanisms by which TnC and TnR affect neuronal differentiation are not well understood. In this study, we hypothesized that different forms of tenascin might regulate the neural transdifferentiation of human bone marrow-derived mesenchymal stem cells. Human MSCs were cultured in media incorporated with soluble tenascins, or on precoated tenascins. In a qualitative polymerase chain reaction analysis, adding a soluble TnC and TnR mixture to the medium significantly enhanced the expression of neuronal and glial markers, whereas no synaptic markers were expressed. Conversely, in groups of cells treated with coated TnC, hMSCs showed neurite outgrowth and synaptic marker expression. After being treated with coated TnR, hMSCs exhibited neuronal differentiation; however, it inhibited neurite outgrowth and synaptic marker expression. A combination of TnC and TnR significantly promoted hMSC differentiation in neurons or oligodendrocytes, induced neurite formation, and inhibited differentiation into astrocytes. Furthermore, the effect of the tenascin mixture showed dose-dependent effects, and a mixture ratio of 1:1 to 1:2 (TnC:TnR) provided the most obvious differentiation of neurons and oligodendrocytes. In a functional blocking study, integrin alpha7 and alpha9beta1-blocking antibodies inhibited, respectively, 80% and 20% of mRNA expression by hMSCs in the coated tenascin mixture. In summary, the coated combination of TnC and TnR appeared to regulate neural differentiation signaling through integrin alpha7 and alpha9beta1 in bone marrow-derived hMSCs. Our findings demonstrate novel mechanisms by which tenascin regulates neural differentiation, and enable the use of cell therapy to treat neurodegenerative diseases.

Ubil, E., J. Duan, et al. "Mesenchymal-endothelial transition contributes to cardiac neovascularization." Nature **514**(7524): 585-90.

Endothelial cells contribute to a subset of cardiac fibroblasts by undergoing endothelial-to-mesenchymal transition, but whether cardiac fibroblasts can adopt an endothelial cell fate and directly contribute to neovascularization after cardiac injury is not known. Here, using genetic fate map techniques, we demonstrate that cardiac fibroblasts rapidly adopt an endothelial-cell-like phenotype after acute ischaemic cardiac injury. Fibroblast-derived

endothelial cells exhibit anatomical and functional characteristics of native endothelial cells. We show that the transcription factor p53 regulates such a switch in cardiac fibroblast fate. Loss of p53 in cardiac fibroblasts severely decreases the formation of fibroblast-derived endothelial cells, reduces post-infarct vascular density and worsens cardiac function. Conversely, stimulation of the p53 pathway in cardiac fibroblasts augments mesenchymal-to-endothelial transition, enhances vascularity and improves cardiac function. These observations demonstrate that mesenchymal-to-endothelial transition contributes to neovascularization of the injured heart and represents a potential therapeutic target for enhancing cardiac repair.

Ullah, M., M. Sittinger, et al. "Transdifferentiation of adipogenically differentiated cells into osteogenically or chondrogenically differentiated cells: phenotype switching via dedifferentiation." Int J Biochem Cell Biol **46**: 124-37.

Reprogramming is a new wave in cellular therapies to achieve the vital goals of regenerative medicine. Transdifferentiation, whereas the differentiated state of cells could be reprogrammed into other cell types, meaning cells are no more locked in their differentiated circle. Hence, cells of choice from abundant and easily available sources such as fibroblast and adipose tissue could be converted into cells of demand, to restore the diseased tissues. Before diverting this new approach into effective clinical use, transdifferentiation could not be simply overlooked, as it challenges the normal paradigms of biological laws, where mature cells transdifferentiate not only within same germ layers, but even across the lineage boundaries. How unipotent differentiated cells reprogram into another, and whether transdifferentiation proceeds via a direct cell-to-cell conversion or needs dedifferentiation. To address such questions, MSC were adipogenically differentiated followed by direct transdifferentiation, and subsequently examined by histology, immunohistochemistry, qPCR and single cell analysis. Direct cellular conversion of adipogenic lineage cells into osteogenic or chondrogenic resulted in mixed culture of both lineage cells (adipogenic and new acquiring osteogenic/chondrogenic phenotypes). On molecular level, such conversion was confirmed by significantly upregulated expression of PPARG, FABP4, SPP1 and RUNX2. Chondrogenic transdifferentiation was verified by significantly upregulated expression of PPARG, FABP4, SOX9 and COL2A1. Single cell analysis did not support the direct cell-to-cell conversion, rather described the involvement of dedifferentiation. Moreover, some differentiated single cells did not change their phenotype and were resistant to transdifferentiation, suggesting that differentiated cells behave differently during cellular conversion. An obvious characterization of differentiated cells could be helpful to understand the process of transdifferentiation.

Vainshtein, J. M., R. Kabarriti, et al. "Bone marrow-derived stromal cell therapy in cirrhosis: clinical evidence, cellular mechanisms, and implications for the treatment of hepatocellular carcinoma." Int J Radiat Oncol Biol Phys **89**(4): 786-803.

Current treatment options for hepatocellular carcinoma (HCC) are often limited by the presence of underlying liver disease. In patients with liver cirrhosis, surgery, chemotherapy, and radiation therapy all carry a high risk of hepatic complications, ranging from ascites to fulminant liver failure. For patients receiving radiation therapy, cirrhosis dramatically reduces the already limited radiation tolerance of the liver and represents the most important clinical risk factor for the development of radiation-induced liver disease. Although improvements in conformal radiation delivery techniques have improved our ability to safely irradiate confined areas of the liver to increasingly higher doses with excellent local disease control, patients with moderate-to-severe liver cirrhosis continue to face a shortage of treatment options for HCC. In recent years, evidence has emerged supporting the use of bone marrow-derived stromal cells (BMSCs) as a promising treatment for liver cirrhosis, with several clinical studies demonstrating sustained improvement in clinical parameters of liver function after autologous BMSC infusion. Three predominant populations of BMSCs, namely hematopoietic stem cells, mesenchymal stem cells, and endothelial progenitor cells, seem to have therapeutic potential in liver injury and cirrhosis. Preclinical studies of BMSC transplantation have identified a range of mechanisms through which these cells mediate their therapeutic effects, including hepatocyte transdifferentiation and fusion, paracrine stimulation of hepatocyte proliferation, inhibition of activated hepatic stellate cells, enhancement of fibrolytic matrix metalloproteinase activity, and neovascularization of regenerating liver. By bolstering liver function in patients with underlying Child's B or C cirrhosis, autologous BMSC infusion holds great promise as a therapy to improve the safety, efficacy, and utility of surgery, chemotherapy, and hepatic radiation therapy in the treatment of HCC.

Vega, M. E., V. Giroux, et al. "Inhibition of Notch signaling enhances transdifferentiation of the esophageal squamous epithelium towards a Barrett's-

like metaplasia via KLF4." Cell Cycle **13**(24): 3857-66.

Barrett's esophagus (BE) is defined as an incomplete intestinal metaplasia characterized generally by the presence of columnar and goblet cells in the formerly stratified squamous epithelium of the esophagus. BE is known as a precursor for esophageal adenocarcinoma. Currently, the cell of origin for human BE has yet to be clearly identified. Therefore, we investigated the role of Notch signaling in the initiation of BE metaplasia. Affymetrix gene expression microarray revealed that BE samples express decreased levels of Notch receptors (NOTCH2 and NOTCH3) and one of the the ligands (JAG1). Furthermore, BE tissue microarray showed decreased expression of NOTCH1 and its downstream target HES1. Therefore, Notch signaling was inhibited in human esophageal epithelial cells by expression of dominant-negative-Mastermind-like (dnMAML), in concert with MYC and CDX1 overexpression. Cell transdifferentiation was then assessed by 3D organotypic culture and evaluation of BE-lineage specific gene expression. Notch inhibition promoted transdifferentiation of esophageal epithelial cells toward columnar-like cells as demonstrated by increased expression of columnar keratins (K8, K18, K19, K20) and glandular mucins (MUC2, MUC3B, MUC5B, MUC17) and decreased expression of squamous keratins (K5, K13, K14). In 3D culture, elongated cells were observed in the basal layer of the epithelium with Notch inhibition. Furthermore, we observed increased expression of KLF4, a potential driver of the changes observed by Notch inhibition. Interestingly, knockdown of KLF4 reversed the effects of Notch inhibition on BE-like metaplasia. Overall, Notch signaling inhibition promotes transdifferentiation of esophageal cells toward BE-like metaplasia in part via upregulation of KLF4. These results support a novel mechanism through which esophageal epithelial transdifferentiation promotes the evolution of BE.

Venkatesh, K., L. Srikanth, et al. "In vitro transdifferentiation of human cultured CD34+ stem cells into oligodendrocyte precursors using thyroid hormones." Neurosci Lett **588**: 36-41.

The extent of myelination on the axon promotes transmission of impulses in the neural network, any disturbances in this process results in the neurodegenerative condition. Transplantation of oligodendrocyte precursors that supports in the regeneration of axons through myelination is an important step in the restoration of damaged neurons. Therefore, in the present study, the differentiation of human CD34+ stem cells into oligodendrocytes was carried out. The pure human CD34+ culture developed from the stem cells obtained from a peripheral blood of a donor were subjected to oligodendrocyte differentiation medium (ODM). The ODM at a concentration of 40ng/ml thyroxine, 40ng/ml 3,3',5-tri-iodo-thyronine showed distinct morphological changes from day 6 to 9 with cells exhibiting conspicuous stellate morphology and extensive foot processes. The real-time PCR analysis showed prominent expression of Olig2, CNPase, PDGFRalpha and PLP1/DM20 in the differentiated cells confirming the formed cells are oligodendrocyte precursors. The expression of these genes increased from days 6 to 9 corresponding to the morphological changes observed with almost no expression of GFAP+ cells. The distinct CNPase activity was observed in these differentiated cells compared to normal CD34+ stem cells correlating with results of real-time PCR conclusively explains the development of oligodendrocytes from human CD34+ stem cells.

Weissbein, U., U. Ben-David, et al. "Virtual karyotyping reveals greater chromosomal stability in neural cells derived by transdifferentiation than those from stem cells." Cell Stem Cell **15**(6): 687-91.

Neural cells can be derived either from pluripotent or adult stem cells via differentiation or by transdifferentiation from other cell types with the aid of tissue regulators. We compared the chromosomal stability of over 500 neural cell samples from human and mouse with virtual karyotyping (e-karyotyping). We detected notable genomic instability in cells derived from pluripotent or adult stem cells, but surprisingly, transdifferentiated cells seemed more chromosomally stable, except if they were reprogrammed using pluripotency factors.

Wu, H. and R. I. Mahato "Mesenchymal stem cell-based therapy for type 1 diabetes." Discov Med **17**(93): 139-43.

Diabetes has increasingly become a worldwide health problem, causing huge burden on healthcare system and economy. Type 1 diabetes (T1D), traditionally termed "juvenile diabetes" because of an early onset age, is affecting 5-10% of total diabetic population. Insulin injection, the predominant treatment for T1D, is effective to ameliorate the hyperglycemia but incompetent to relieve the autoimmunity and to regenerate lost islets. Islet transplantation, an experimental treatment for T1D, also suffers from limited supply of human islets and poor immunosuppression. The recent progress in regenerative medicine, especially stem cell therapy, has suggested several novel and potential cures for T1D. Mesenchymal stem cell (MSC) based cell therapy is among one of them. MSCs are a type of adult stem cells residing in bone marrow, adipose

tissue, umbilical cord blood, and many other tissues. MSCs, with self-renewal potential and transdifferentiation capability, can be expanded in vitro and directed to various cell lineages with relatively less efforts. MSCs have well-characterized hypoimmunogenicity and immunomodulatory effect. All these features make MSCs attractive for treating T1D. Here, we review the properties of MSCs and some of the recent progress using MSCs as a new therapeutic in the treatment of T1D. We also discuss the strength and limitations of using MSC therapy in human trials.

Xuan, Z. X., L. N. Li, et al. "Fully human VEGFR2 monoclonal antibody BC001 attenuates tumor angiogenesis and inhibits tumor growth." Int J Oncol **45**(6): 2411-20.

The critical role of VEGFR2 in tumor neovascularization and progression has allowed the design of clinically beneficial therapies based on it. Here we show that BC001, a new fully human anti-VEGFR2 monoclonal antibody, inhibits VEGF-stimulated endothelial cell migration, tube formation, and effectively suppressed the transdifferentiation of cancer stem cells into endothelial cells in vitro. Since BC001 exhibited no activity against the mouse VEGFR2 and mouse based study was required to confirm its efficacy in vivo, BC101, the mouse analogue of BC001, was developed. BC101 significantly attenuated angiogenesis according to Matrigel plug assay and resulted in ~80% growth inhibition of mouse B16F10 homograft tumors relative to vehicle control. Similarly, human analogue BC001 suppressed the growth of human xenograft tumors HCT116 and BGC823.

Yu, J., Y. K. Tu, et al. "Stemness and transdifferentiation of adipose-derived stem cells using L-ascorbic acid 2-phosphate-induced cell sheet formation." Biomaterials **35**(11): 3516-26.

Cell sheet technology has emerged as an important tissue engineering approach. Adipose-derived stem cells (ASCs) have valuable applications in regenerative medicine, but their stemness and differentiation capabilities in the cell sheet format have not been well investigated. In this study, we found that l-ascorbate 2-phosphate (A2-P), a stable form of ascorbic acid, significantly enhanced ASC proliferation and induced ASC sheet fabrication in 7 days with abundant extracellular matrix deposition. Importantly, A2-P treatment significantly enhanced expression of pluripotent markers Sox-2, Oct-4 and Nanog, but treating ASCs with antioxidants other than A2-P revealed no stemness enhancement. Moreover, ASC treatment with A2-P and a collagen synthesis inhibitor, L-2-azetidine carboxylic acid or cis-4-hydroxy-d-proline, significantly inhibited the A2-P-enhanced expression of stemness markers. These findings demonstrated that A2-P enhances stemness of ASCs through collagen synthesis and cell sheet formation. We also showed that A2-P-stimulated collagen synthesis in ASCs may be mediated through ERK1/2 pathway. By culturing the ASC sheets in proper induction media, ASC transdifferentiation capabilities into neuron and hepatocyte-like cells were significantly enhanced after cell sheet formation, while adipogenic and osteogenic differentiation capacities were still maintained. Using a murine model of healing-impaired cutaneous wound, faster wound healing was noted in the group that received ASC sheet treatment, and we observed significantly more engrafted ASCs with evidence of differentiation toward endothelial and epidermal lineages in the cutaneous wound tissue. Therefore, A2-P-mediated ASC sheet formation enhanced ASC stemness and transdifferentiation capabilities, thereby representing a promising approach for applications in regenerative medicine.

Yuan, W., W. Liu, et al. "Effects of BMSCs interactions with adventitial fibroblasts in transdifferentiation and ultrastructure processes." Int J Clin Exp Pathol **7**(7): 3957-65.

In this study an in vitro model of simulated blood vessel injury was used to study the effects of bone marrow-derived mesenchymal stem cells (BMSCs) morphology and to detect vascular smooth muscle actin (SM alpha-actin) expression in the presence of adventitial fibroblasts. BMSCs from rats with DAPI-labeled nuclei were co-cultured with adventitial fibroblasts for 7 days, while BMSCs cultured alone served as controls. Cell morphology of BMSCs was assessed by laser confocal microscopy and SM alpha-actin or calponin expression in BMSCs was detected by immunofluorescence staining. The expression of SM alpha-actin mRNA was identified using RT-PCR. Cell ultrastructure was assessed by electron microscopy. The results demonstrate that BMSCs with DAPI-labeled nuclei were smaller compared with fibroblasts, and their nuclei emitted a blue fluorescence. Most BMSCs displayed a polygonal shape changing from their original long fusiform shape. BMSCs with blue nuclei and red cytoplasm (SM alpha-actin positive or calponin positive) were observed, and a substantial number of filaments were present in the cytoplasm as observed under electron microscopy. The number of these cells increased as a function of culture duration. However, SM alpha-actin expression was weak and calponin expression was not detected in the control group. This study provides important new information on the characterization of artherosclerosis pathogenesis and vascular restenosis

after blood vessel injury. Our findings demonstrate that direct interactions with adventitial fibroblasts can induce vascular smooth muscle-like cell differentiation in BMSCs.

Zhang, H., X. Wang, et al. "Time point-based integrative analyses of deep-transcriptome identify four signal pathways in blastemal regeneration of zebrafish lower jaw." Stem Cells 2015;**33**(3): 806-18.

There has been growing interest in applying tissue engineering to stem cell-based regeneration therapies. We have previously reported that zebrafish can faithfully regenerate complicated tissue structures through blastemal cell type conversions and tissue reorganization. To unveil the regenerative factors and engineering arts of blastemal regeneration, we conducted transcriptomal analyses at four time points corresponding to preamputation, re-epitheliation, blastemal formation, and respecification. By combining the hierarchical gene ontology term network, the DAVID annotation system, and Euclidean distance clustering, we identified four signaling pathways: foxi1-foxo1b-pou3f1, pax3a-mant3a-col11/col2, pou5f1-cdx4-kdrl, and isl1-wnt11 PCP-sox9a. Results from immunohistochemical staining and promoter-driven transgenic fish suggest that these pathways, respectively, define wound epidermis reconstitution, cell type conversions, blastemal angiogenesis/vasculogenesis, and cartilage matrix-orientation. Foxi1 morpholino-knockdown caused expansions of Foxo1b- and Pax3a-expression in the basal layer-blastemal junction region. Moreover, foxi1 morphants displayed increased sox9a and hoxa2b transcripts in the embryonic pharyngeal arches. Thus, a Foxi1 signal switch is required to establish correct tissue patterns, including re-epitheliation and blastema formation. This study provides novel insight into a blastema regeneration strategy devised by epithelial cell transdifferentiation, blood vessel engineering, and cartilage matrix deposition.

Zhang, Q., H. Wang, et al. "Atorvastatin treatment improves the effects of mesenchymal stem cell transplantation on acute myocardial infarction: the role of the RhoA/ROCK/ERK pathway." Int J Cardiol **176**(3): 670-9.

BACKGROUND: Statins protect mesenchymal stem cells (MSCs) against the harsh microenvironment and improve the efficacy of MSC transplantation after acute myocardial infarction (AMI); however, the mechanism remains uncertain. Furthermore, the transdifferentiation potential of MSCs in the post-infarct heart remains highly controversial. The RhoA/Rho-associated coiled-coil-forming kinase (ROCK) pathway participates in many aspects of the damaged heart after AMI and related to the "pleiotropic" effects of statins. This study aimed to explore whether atorvastatin (ATV) facilitates the survival and therapeutic efficacy of MSCs via the inhibition of RhoA/ROCK pathway and subsequently its downstream molecular extracellular regulated protein kinase (ERK1/2), and to investigate the transdifferentiation potential of MSCs in vivo. METHODS AND RESULTS: Female rats received myocardial injections of male rat MSCs 30 min after AMI. Four weeks after AMI, ATV combined with MSC treatment resulted in improved cardiac function and reduced infarct area. ATV facilitated the MSC survival, as revealed by the increased expression of Y chromosomal genes and the increased number of Y chromosome-positive cells; however, no transdifferentiation markers were observed. ATV inhibited the production of inflammatory cytokines both in vitro and vivo, accompanied by suppression of ROCK and ERK activities. Geranylgeranyl pyrophosphate (GGPP) abrogated the effects of ATV in the H9c2 cells under hypoxia/serum deprivation (H/SD), while the ROCK inhibitor fasudil mimicked the benefits of ATV after AMI. CONCLUSIONS: ATV improves the post-infarct microenvironment via RhoA/ROCK/ERK inhibition and thus facilitates the survival and efficacy of implanted MSCs. Transdifferentiation may be not responsible for the cardiac benefits that follow MSC transplantation.

Zuryn, S., A. Ahier, et al. "Transdifferentiation. Sequential histone-modifying activities determine the robustness of transdifferentiation." Science **345**(6198): 826-9.

Natural interconversions between distinct somatic cell types have been reported in species as diverse as jellyfish and mice. The efficiency and reproducibility of some reprogramming events represent unexploited avenues in which to probe mechanisms that ensure robust cell conversion. We report that a conserved H3K27me3/me2 demethylase, JMJD-3.1, and the H3K4 methyltransferase Set1 complex cooperate to ensure invariant transdifferentiation (Td) of postmitotic Caenorhabditis elegans hindgut cells into motor neurons. At single-cell resolution, robust conversion requires stepwise histone-modifying activities, functionally partitioned into discrete phases of Td through nuclear degradation of JMJD-3.1 and phase-specific interactions with transcription factors that have conserved roles in cell plasticity and terminal fate selection. Our results draw parallels between epigenetic mechanisms underlying robust Td in nature and efficient cell reprogramming in vitro.

Turritopsis nutricula is a hydrozoan that can revert to the sexually immature (polyp stage) after

becoming sexually mature. It is the only known metazoan capable of reverting completely to a sexually immature, colonial stage after having reached sexual maturity as a solitary stage. It does this through the cell development process of transdifferentiation. This cycle can repeat indefinitely that offers it biologically immortal. To study the reason of the biological immortality of *Turritopsis nutricula* possibly supplies the way finding the biological immortality for human. Immortality has been a subject of fascination to humanity since at least the beginning of history and has been a major point of focus of religion, as well as the subject of speculation, fantasy, and debate (Ma and Yang, 2010).

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