Klf4 and stem cell literatures

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Abstract: Kruppel-like factor 4 (Klf4) is a member of the KLF family of transcription factors and regulates proliferation, differentiation, apoptosis and somatic cell reprogramming. In embryonic stem (ES) cells, Klf4 can be a good indicator of stem-like capacity. Klf4 is a 55 kD transcription factor containing three zinc finger domains. Klf4 binds the CACCC core sequence at multiple sites in the 5' flanking region of the gene and can activate its transcription. It interacts with the c-terminal domain of MUC1 to enhance the suppression of TP53/p53 tran-scription. The Klf4 gene is conserved in chimpanzee, Rhesus monkey, dog, cow, mouse, rat, chicken, zebrafish, and frog, etc.
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1. Introduction

Kruppel-like factor 4 (Klf4) is a member of the KLF family of transcription factors and regulates proliferation, differentiation, apoptosis and somatic cell reprogramming. In embryonic stem (ES) cells, Klf4 can be a good indicator of stem-like capacity.

Klf4 is a 55 kD transcription factor containing three zinc finger domains. Klf4 binds the CACCC core sequence at multiple sites in the 5' flanking region of the gene and can activate its transcription. It interacts with the c-terminal domain of MUC1 to enhance the suppression of TP53/p53 tran-scription. The Klf4 gene is conserved in chimpanzee, Rhesus monkey, dog, cow, mouse, rat, chicken, zebrafish, and frog, etc. KLF4 is an oncogene or tumor suppressor in different tumor types.

Klf 4 and 5 are two closely related members of the Klf family playing key roles in cell cycle regulation, somatic cell reprogramming and pluripotency. Klf4 and Klf5 differentially inhibit mesoderm and endoderm differentiation in ES cells.

There are many methods to deliver the transcription factors into target cells to generate iPSCs. The first method is retrovirus or lentivirus transduction. The problem of this technique is the genome integration of virus DNA which could possibly alter differentiation potential or other malignant transformation. The second method is adenoviral vectors to induce iPSC. The advantage of adenovirus vector based expression is that the transgenes will not integrate into the house genome, thus reduces the risk of tumorogenesis. The third one is a plasmid based transfection that can avoid the genome integration also. Recently, the Cre-recombinase excisable systems are used in iPSC induction and subsequent transgene removal making the iPSC technology closer to clinic applications.

Literatures

The following gives some recent reference papers on Klf4.

Aksoy, I., V. Giudice, et al. "Klf4 and Klf5 differentially inhibit mesoderm and endoderm differentiation in embryonic stem cells." Nat Commun. 2014 Apr 28;5:3719. doi: 10.1038/ncomms4719.

Kruppel-like factors (Klf) 4 and 5 are two closely related members of the Klf family, known to play key roles in cell cycle regulation, somatic cell reprogramming and pluripotency. Here we focus on the functional divergence between Klf4 and Klf5 in the inhibition of mouse embryonic stem (ES) cell differentiation. Using microarrays and chromatin immunoprecipitation coupled to ultra-high-throughput DNA sequencing, we show that Klf4 negatively regulates the expression of endodermal markers in the undifferentiated ES cells, including transcription factors involved in the commitment of pluripotent stem cells to endoderm differentiation. Knockdown of Klf4 enhances differentiation towards visceral and definitive endoderm. In contrast, Klf5 negatively regulates the expression of mesodermal markers, some of which control commitment to the mesoderm lineage, and knockdown of Klf5 specifically enhances differentiation towards mesoderm. We conclude that Klf4 and Klf5 differentially inhibit mesoderm and endoderm differentiation in murine ES cells.

Alisoltani, A., H. Fallahi, et al. "Prediction of potential cancer-risk regions based on transcriptome data: towards a comprehensive view." PLoS One. 2014 May 5;9(5):e96320. doi: 10.1371/journal.pone.0096320. eCollection 2014.

A novel integrative pipeline is presented for discovery of potential cancer-susceptibility regions (PCSRs) by calculating the number of altered genes at each chromosomal region, using expression microarray datasets of different human cancers (HCs). Our novel approach comprises primarily predicting PCSRs followed by identification of key genes in these regions to obtain potential regions harboring new cancer-associated variants. In addition to finding new cancer causal variants, another advantage in prediction of such risk regions is simultaneous study of different types of genomic variants in line with focusing on specific chromosomal regions. Using this pipeline we extracted numbers of regions with highly altered expression levels in cancer condition. Regulatory networks were also constructed for different types of cancers following the identification of altered mRNA and microRNAs. Interestingly, results showed that GAPDH, LIFR, ZEB2, mir-21, mir-30a, mir-141 and mir-200c, all located at PCSRs, are common

altered factors in constructed networks. We found a number of clusters of altered mRNAs and miRNAs on predicted PCSRs (e.g.12p13.31) and their common regulators including KLF4 and SOX10. Large scale prediction of risk regions based on transcriptome data can open a window in comprehensive study of cancer risk factors and the other human diseases.

Almeida-Suhett, C. P., Z. Li, et al. "Temporal course of changes in gene expression suggests a cytokine-related mechanism for long-term hippocampal alteration after controlled cortical impact." J Neurotrauma. 2014 Apr 1;31(7):683-90. doi: 10.1089/neu.2013.3029. Epub 2014 Mar 3.

Mild traumatic brain injury (mTBI) often has long-term effects on cognitive function and social behavior. Altered gene expression may be predictive of long-term psychological effects of mTBI, even when acute clinical effects are minimal or transient. Controlled cortical impact (CCI), which causes concussive, but nonpenetrant, trauma to underlying (non-cortical) brain, resulting in persistent changes in hippocampal synaptic function, was used as a model of mTBI. The hippocampal transcriptomes of sham-operated or injured male rats at 1, 7, and 30 days postinjury were examined using microarrays comprising a comprehensive set of expressed genes, subsequently confirmed by quantitative reverse-transcriptase polymerase chain reaction. Transcripts encoding the chemokines, chemokine (C-C motif) ligand (Ccl)2 and Ccl7, inflammatory mediators lipocalin-2 (Lcn2) and tissue inhibitor of metalloproteinase 1 (Timp1), immunocyte activators C-C chemokine receptor type 5 (Ccr5) and Fc fragment of IgG, low affinity IIb, receptor (CD32) (Fcgr2b), the major histocompatibility complex II immune response-related genes, Cd74 and RT1 class II, locus Da (RT1-Da), the complement component, C3, and the transcription factor, Kruppel-like factor 4 (Klf4), were identified as early (Ccl2, Ccl7, Lcn2, and Timp1), intermediate (Ccr5, Fcgr2b, Cd74, RT1-Da, and C3), and late (Klf4) markers for bilateral hippocampal response to CCI. Ccl2 and Ccl7 transcripts were up-regulated within 24 h after CCI, and their elevation subsided within 1 week of injury. Other transcriptional changes occurred later and were more stable, some persisting for at least 1 month, suggesting that short-term inflammatory responses trigger longer-term alteration in the expression of genes previously associated with injury, aging, and neuronal function in the brain. These transcriptional responses to mTBI may underlie long-term changes in excitatory and inhibitory neuronal imbalance in hippocampus, leading to long-term behavioral consequences of mTBI.

Bae, K. S., S. Y. Kim, et al. "Identification of lactoferrin as a human dedifferentiation factor through the studies of reptile tissue regeneration mechanisms." J Microbiol Biotechnol. 2014 Jun 28;24(6):869-78.

In this study, we performed two-dimensional electrophoresis with protein extracts from lizard tails, and analyzed the protein expression profiles during the tissue regeneration to identify the dedifferentiation factor. As a result, we identified 18 protein spots among total of 292 spots, of which proteins were specifically expressed during blastema formation. We selected lactoferrin as a candidate because it is the mammalian homolog of leech-derived tryptase inhibitor, which showed the highest frequency among the 18 proteins. Lactoferrin was specifically expressed in various stem cell lines, and enhanced the efficiency of iPSC generation upto approximately 7-fold relative to the control. Furthermore, lactoferrin increased the efficiency by 2-fold without enforced expression of Klf4. These results suggest that lactoferrin may induce dedifferentiation, at least partly by increasing the expression of Klf4.

Baranov, P. Y., B. A. Tucker, et al. "Low-oxygen culture conditions extend the multipotent properties of human retinal progenitor cells." Tissue Eng Part A. 2014 May;20(9-10):1465-75. doi: 10.1089/ten.TEA.2013.0361. Epub 2014 Jan 24.

PURPOSE: Development of an effective cell-based therapy is highly dependent upon having a reproducible cell source suitable for transplantation. One potential source, isolated from the developing fetal neural retina, is the human retinal progenitor cell (hRPC). One limiting factor for the use of hRPCs is their in vitro expansion limit. As such, the aim of this study was to determine whether culturing hRPCs under 3% O2 would support their proliferative capacity while maintaining multipotency. METHODS: To determine the effect of low oxygen on the ability of hRPCs to self-renew, rates of proliferation and apoptosis, telomerase activity, and expression of proliferative, stemness, and differentiation markers were assessed for hRPCs cultured in 3% and 20% oxygen conditions. RESULTS: Culture under 3% oxygen increases the proliferation rate and shifts the proliferation limit of hRPCs to greater 40 divisions. This increased capacity for proliferation is correlated with an upregulation of Ki67, CyclinD1, and telomerase activity and a decrease in p53 expression and apoptosis. Increased expression of cMyc, Klf4, Oct4, and Sox2 in 3% O(2) is correlated with stabilization of both HIF1alpha and HIF2alpha. The eye field development markers Pax6, Sox2, and Otx2 are present in hRPCs up to passage 16 in 3% O(2) . Following in vitro differentiation hRPCs expanded in the 3% O(2) were able to generate specialized retinal cells, including rods and cones. CONCLUSIONS: Low-oxygen culture conditions act to maintain both multipotency and self-renewal properties of hRPCs in vitro. The extended expansion limits permit the development of a clinical-grade reagent for transplantation.

Brandl, C., S. J. Zimmermann, et al. "In-Depth Characterisation of Retinal Pigment Epithelium (RPE) Cells Derived from Human Induced Pluripotent Stem Cells (hiPSC)." Neuromolecular Med. 2014 Sep;16(3):551-64. doi: 10.1007/s12017-014-8308-8. Epub 2014 May 7.

Induced pluripotent stem cell (iPSC)-derived retinal pigment epithelium (RPE) has widely been appreciated as a promising tool to model human ocular disease emanating from primary RPE pathology. Here, we describe the successful reprogramming of adult human dermal fibroblasts to iPSCs and their differentiation to pure expandable RPE cells with structural and functional features characteristic for native RPE. Fibroblast cultures were established from skin biopsy material and subsequently reprogrammed following polycistronic lentiviral transduction with OCT4, SOX2, KLF4 and L-Myc. Fibroblast-derived iPSCs showed typical morphology, chromosomal integrity and a distinctive stem cell marker profile. Subsequent differentiation resulted in expandable pigmented hexagonal RPE cells. The cells revealed stable RNA expression of mature RPE markers RPE65, RLBP and BEST1. Immunolabelling verified localisation of BEST1 at the basolateral plasma membrane, and scanning electron microscopy showed typical microvilli at the apical side of iPSC-derived RPE cells. Transepithelial resistance was maintained at high levels during cell culture indicating functional formation of tight junctions. Secretion capacity was demonstrated for VEGF-A. Feeding of porcine photoreceptor outer segments revealed the proper ability of these cells for phagocytosis. IPSC-derived RPE cells largely maintained these properties after cryopreservation. Together, our study underlines that adult dermal fibroblasts can serve as a valuable resource for iPSC-derived RPE with characteristics highly reminiscent of true RPE cells. This will allow its broad application to establish cellular models for RPE-related human diseases.

Buganim, Y., S. Markoulaki, et al. "The Developmental Potential of iPSCs Is Greatly Influenced by Reprogramming Factor Selection." Cell Stem Cell. 2014 Sep 4;15(3):295-309. doi: 10.1016/j.stem.2014.07.003.

Induced pluripotent stem cells (iPSCs) are commonly generated by transduction of Oct4, Sox2, Klf4, and Myc (OSKM) into cells. Although iPSCs are pluripotent, they frequently exhibit high variation in terms of quality, as measured in mice by chimera

contribution and tetraploid complementation. Reliably high-quality iPSCs will be needed for future therapeutic applications. Here, we show that one major determinant of iPSC quality is the combination of reprogramming factors used. Based on tetraploid complementation, we found that ectopic expression of Sall4, Nanog, Esrrb, and Lin28 (SNEL) in mouse embryonic fibroblasts (MEFs) generated high-quality iPSCs more efficiently than other combinations of factors including OSKM. Although differentially methylated regions, transcript number of master regulators, establishment of specific superenhancers, and global aneuploidy were comparable between high- and low-quality lines, aberrant gene expression, trisomy of chromosome 8, and abnormal H2A.X deposition were distinguishing features that could potentially also be applicable to human.

Carter, A. C., B. N. Davis-Dusenbery, et al. "Nanog-Independent Reprogramming to iPSCs with Canonical Factors." Stem Cell Reports. 2014 Jan 31;2(2):119-26. doi: 10.1016/j.stemcr.2013.12.010. eCollection 2014 Feb 11.

It has been suggested that the transcription factor Nanog is essential for the establishment of pluripotency during the derivation of embryonic stem cells and induced pluripotent stem cells (iPSCs). However, successful reprogramming to pluripotency with a growing list of divergent transcription factors, at ever-increasing efficiencies, suggests that there may be many distinct routes to a pluripotent state. Here, we have investigated whether Nanog is necessary for reprogramming murine fibroblasts under highly efficient conditions using the canonical-reprogramming factors Oct4, Sox2, Klf4, and cMyc. In agreement with prior results, the efficiency of reprogramming Nanog (-/-) fibroblasts was significantly lower than that of control fibroblasts. However, in contrast to previous findings, we were able to reproducibly generate iPSCs from Nanog (-/-) fibroblasts that effectively contributed to the germline of chimeric mice. Thus, whereas Nanog may be an important mediator of reprogramming, it is not required for establishing pluripotency in the mouse, even under standard conditions.

Chakraborty, C., S. R. S, et al. "Network analysis of transcription factors for nuclear reprogramming into induced pluripotent stem cell using bioinformatics." Cell J. 2014 Winter;15(4):332-9. Epub 2013 Nov 20.

OBJECTIVE: Research related to induce pluripotent stem (iPS) cell generation has increased rapidly in recent years. Six transcription factors, namely OCT4, SOX2, C-MYC, KLF4, NANOG, and LIN28 have been widely used for iPS cell generation. As there is a lack of data on intra- and inter-networking among these six different transcription factors, the objective of this study is to analyze the intra- and inter-networks between them using bioinformatics. MATERIALS AND METHODS: In this computational biology study, we used AminoNet, MATLAB to examine networking between the six different transcription factors. The directed network was constructed using MATLAB programming and the distance between nodes was estimated using a phylogram. The protein-protein interactions between the nuclear reprogramming factors was performed using the software STRING. RESULTS: The relationship between C-MYC and NANOG was depicted using a phylogenetic tree and the sequence analysis showed OCT4, C-MYC, NANOG, and SOX2 together share a common evolutionary origin. CONCLUSION: This study has shown an innovative rapid method for the analysis of intra and inter-networking among nuclear reprogramming factors. Data presented may aid researchers to understand the complex regulatory networks involving iPS cell generation.

Chang, Q., B. Chen, et al. "Arsenic-induced sub-lethal stress reprograms human bronchial epithelial cells to CD61 cancer stem cells." Oncotarget. 2014 Mar 15;5(5):1290-303.

In the present report, we demonstrate that sub-lethal stress induced by consecutive exposure to 0.25 microM arsenic (As3+) for six months can trigger reprogramming of the human bronchial epithelial cell (BEAS-2B) to form cancer stem cells (CSCs) without forced introduction of the stemness transcription factors. These CSCs formed from As3+-induced sub-lethal stress featured with an increased expression of the endogenous stemness genes, including Oct4, Sox2, Klf4, Myc, and others that are associated with the pluripotency and self-renewal of the CSCs. Flow cytometry analysis indicated that 90% of the CSC cells are CD61 , whereas 100% of the parental cells are CD61+. These CD61 CSCs are highly tumorigenic and metastatic to the lung in xenotransplantation tests in NOD/SCID Il2rgamma-/- mice. Additional tests also revealed that the CD61 CSCs showed a significant decrease in the expression of the genes important for DNA repair and oxidative phosphorylation. To determine the clinical relevance of the above findings, we stratified human lung cancers based on the level of CD61 protein and found that CD61low cancer correlates with poorer survival of the patients. Such a correlation was also observed in human breast cancer and ovarian cancer. Taken together, our findings suggest that in addition to the traditional approaches of enforced introduction of the exogenous stemness circuit transcription factors, sub-lethal stress induced by consecutive low dose As3+ is also able to convert non-stem cells to the CSCs.

Crespin, S., M. Bacchetta, et al. "Cx26 regulates proliferation of repairing basal airway epithelial cells." Int J Biochem Cell Biol. 2014 Jul;52:152-60. doi: 10.1016/j.biocel.2014.02.010. Epub 2014 Feb 22.

The recovery of an intact epithelium following injury is critical for restoration of lung homeostasis, a process that may be altered in cystic fibrosis (CF). In response to injury, progenitor cells in the undamaged areas migrate, proliferate and re-differentiate to regenerate an intact airway epithelium. The mechanisms regulating this regenerative response are, however, not well understood. In a model of circular wound injury of well-differentiated human airway epithelial cell (HAEC) cultures, we identified the gap junction protein Cx26 as an important regulator of cell proliferation. We report that induction of Cx26 in repairing HAECs is associated with cell proliferation. We also show that Cx26 is expressed in a population of CK14-positive basal-like cells. Cx26 silencing in immortalized cell lines using siRNA and in primary HAECs using lentiviral-transduced shRNA enhanced Ki67-labeling index and Ki67 mRNA, indicating that Cx26 acts a negative regulator of HAEC proliferation. Cx26 silencing also markedly decreased the transcription of KLF4 in immortalized HAECs. We further show that CF HAECs exhibited deregulated expression of KLF4, Ki67 and Cx26 as well enhanced rate of wound closure in the early response to injury. These results point to an altered repair process of CF HAECs characterized by rapid but desynchronized initiation of HAEC activation and proliferation.

Csermely, P., J. Hodsagi, et al. "Cancer stem cells display extremely large evolvability: alternating plastic and rigid networks as a potential Mechanism: Network models, novel therapeutic target strategies, and the contributions of hypoxia, inflammation and cellular senescence." Semin Cancer Biol. 2014 Jan 8. pii: S1044-579X(13)00130-2. doi: 10.1016/j.semcancer.2013.12.004.

Cancer is increasingly perceived as a systems-level, network phenomenon. The major trend of malignant transformation can be described as a two-phase process, where an initial increase of network plasticity is followed by a decrease of plasticity at late stages of tumor development. The fluctuating intensity of stress factors, like hypoxia, inflammation and the either cooperative or hostile interactions of tumor inter-cellular networks, all increase the adaptation potential of cancer cells. This may lead to the bypass of cellular senescence, and to the development of cancer stem cells. We propose that the central tenet of cancer stem cell definition lies exactly in the indefinability of cancer stem cells. Actual properties of cancer stem cells depend on the individual "stress-history" of the given tumor. Cancer stem cells are characterized by an extremely

large evolvability (i.e. a capacity to generate heritable phenotypic variation), which corresponds well with the defining hallmarks of cancer stem cells: the possession of the capacity to self-renew and to repeatedly re-build the heterogeneous lineages of cancer cells that comprise a tumor in new environments. Cancer stem cells represent a cell population, which is adapted to adapt. We argue that the high evolvability of cancer stem cells is helped by their repeated transitions between plastic (proliferative, symmetrically dividing) and rigid (quiescent, asymmetrically dividing, often more invasive) phenotypes having plastic and rigid networks. Thus, cancer stem cells reverse and replay cancer development multiple times. We describe network models potentially explaining cancer stem cell-like behavior. Finally, we propose novel strategies including combination therapies and multi-target drugs to overcome the Nietzschean dilemma of cancer stem cell targeting: "what does not kill me makes me stronger".

D'Anselmi, F., M. G. Masiello, et al. "Microenvironment promotes tumor cell reprogramming in human breast cancer cell lines." PLoS One. 2013 Dec 30;8(12):e83770. doi: 10.1371/journal.pone.0083770. eCollection 2013.

The microenvironment drives mammary gland development and function, and may influence significantly both malignant behavior and cell growth of mammary cancer cells. By restoring context, and forcing cells to properly interpret native signals from the microenvironment, the cancer cell aberrant behavior can be quelled, and organization re-established. In order to restore functional and morphological differentiation, human mammary MCF-7 and MDA-MB-231 cancer cells were allowed to grow in a culture medium filled with a 10% of the albumen (EW, Egg White) from unfertilized chicken egg. That unique microenvironment behaves akin a 3D culture and induces MCF-7 cells to produce acini and branching duct-like structures, distinctive of mammary gland differentiation. EW-treated MDA-MB-231 cells developed buds of acini and duct-like structures. Both MCF-7 and MDA-MB-231 cells produced beta-casein, a key milk component. Furthermore, E-cadherin expression was reactivated in MDA-MB-231 cells, as a consequence of the increased cdh1 expression; meanwhile beta-catenin - a key cytoskeleton component - was displaced behind the inner cell membrane. Such modification hinders the epithelial-mesenchymal transition in MDA-MB-231 cells. This differentiating pathway is supported by the contemporary down-regulation of canonical pluripotency markers (Klf4, Nanog). Given that egg-conditioned medium behaves as a 3D-medium, it is likely that cancer phenotype reversion could be ascribed to the changed interactions between cells and their microenvironment.

Davis, M. R., R. Andersson, et al. "Transcriptional profiling of the human fibrillin/LTBP gene family, key regulators of mesenchymal cell functions." Mol Genet Metab. 2014 May;112(1):73-83. doi: 10.1016/j.ymgme.2013.12.006. Epub 2013 Dec 16.

The fibrillins and latent transforming growth factor binding proteins (LTBPs) form a superfamily of extracellular matrix (ECM) proteins characterized by the presence of a unique domain, the 8-cysteine transforming growth factor beta (TGFbeta) binding domain. These proteins are involved in the structure of the extracellular matrix and controlling the bioavailability of TGFbeta family members. Genes encoding these proteins show differential expression in mesenchymal cell types which synthesize the extracellular matrix. We have investigated the promoter regions of the seven gene family members using the FANTOM5 CAGE database for human. While the protein and nucleotide sequences show considerable sequence similarity, the promoter regions were quite diverse. Most genes had a single predominant transcription start site region but LTBP1 and LTBP4 had two regions initiating different transcripts. Most of the family members were expressed in a range of mesenchymal and other cell types, often associated with use of alternative promoters or transcription start sites within a promoter in different cell types. FBN3 was the lowest expressed gene, and was found only in embryonic and fetal tissues. The different promoters for one gene were more similar to each other in expression than to promoters of the other family members. Notably expression of all 22 LTBP2 promoters was tightly correlated and quite distinct from all other family members. We located candidate enhancer regions likely to be involved in expression of the genes. Each gene was associated with a unique subset of transcription factors across multiple promoters although several motifs including MAZ, SP1, GTF2I and KLF4 showed overrepresentation across the gene family. FBN1 and FBN2, which had similar expression patterns, were regulated by different transcription factors. This study highlights the role of alternative transcription start sites in regulating the tissue specificity of closely related genes and suggests that this important class of extracellular matrix proteins is subject to subtle regulatory variations that explain the differential roles of members of this gene family.

Desphande, A., G. Xia, et al. "Methyl-substituted conformationally constrained rexinoid agonists for the retinoid X receptors demonstrate improved efficacy for cancer therapy and prevention." Bioorg Med Chem. 2014 Jan 1;22(1):178-85. doi: 10.1016/j.bmc.2013.11.039. Epub 2013 Dec 1.

(2E,4E,6Z,8Z)-8-(3',4'-Dihydro-1'(2H)-naphthalen-1'-ylidene)-3,7-dimethyl-2,3,6-o ctatrienoinic acid, 9cUAB30, is a selective rexinoid for the retinoid X nuclear receptors (RXR). 9cUAB30 displays substantial chemopreventive capacity with little toxicity and is being translated to the clinic as a novel cancer prevention agent. To improve on the potency of 9cUAB30, we synthesized 4-methyl analogs of 9cUAB30, which introduced chirality at the 4-position of the tetralone ring. The syntheses and biological evaluations of the racemic homolog and enantiomers are reported. We demonstrate that the S-enantiomer is the most potent and least toxic even though these enantiomers bind in a similar conformation in the ligand binding domain of RXR.

Di Stefano, B., J. L. Sardina, et al. "C/EBPalpha poises B cells for rapid reprogramming into induced pluripotent stem cells." Nature. 2014 Feb 13;506(7487):235-9. doi: 10.1038/nature12885. Epub 2013 Dec 15.

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.

Dryden, N. H., L. R. Broome, et al. "Unbiased analysis of potential targets of breast cancer susceptibility loci by Capture Hi-C." Genome Res. 2014 Aug 13. pii: gr.175034.114.

Genome-wide association studies have identified over 70 common variants that are associated with breast cancer risk. Most of these variants map to non-protein-coding regions and several map to gene deserts, regions of several hundred kb lacking protein-coding

genes. We hypothesised that gene deserts harbour long range regulatory elements that can physically interact with target genes to influence their expression. To test this, we developed Capture Hi-C (CHi-C) which, by incorporating a sequence capture step into a Hi-C protocol, allows high resolution analysis of targeted regions of the genome. We used CHi-C to investigate long range interactions at three breast cancer gene deserts mapping to 2q35, 8q24.21 and 9q31.2. We identified interaction peaks between putative regulatory elements ('bait fragments') within the captured regions and 'targets' that included both protein coding genes and long non-coding (lnc)RNAs, over distances of 6.6kb to 2.6Mb. Target protein-coding genes were IGFBP5, KLF4, NSMCE2 and MYC and target lncRNAs included DIRC3, PVT1 and CCDC26. For one gene desert we were able to define two SNPs (rs12613955 and rs4442975) that were highly correlated with the published risk variant and that mapped within the bait end of an interaction peak. In vivo ChIP-qPCR data show that one of these, rs4442975, affects the binding of FOXA1 and implicate this SNP as a putative functional variant.

Durruthy Durruthy, J., C. Ramathal, et al. "Fate of induced pluripotent stem cells following transplantation to murine seminiferous tubules." Hum Mol Genet. 2014 Jun 15;23(12):3071-84. doi: 10.1093/hmg/ddu012. Epub 2014 Jan 20.

Studies of human germ cell development are limited in large part by inaccessibility of germ cells during development. Moreover, although several studies have reported differentiation of mouse and human germ cells from pluripotent stem cells (PSCs) in vitro, differentiation of human germ cells from PSCs in vivo has not been reported. Here, we tested whether mRNA reprogramming in combination with xeno-transplantation may provide a viable system to probe the genetics of human germ cell development via use of induced pluripotent stem cells (iPSCs). For this purpose, we derived integration-free iPSCs via mRNA-based reprogramming with OCT3/4, SOX2, KLF4 and cMYC alone (OSKM) or in combination with the germ cell-specific mRNA, VASA (OSKMV). All iPSC lines met classic criteria of pluripotency. Moreover, global gene expression profiling did not distinguish large differences between undifferentiated OSKM and OSKMV iPSCs; however, some differences were observed in expression of pluripotency factors and germ cell-specific genes, and in epigenetic profiles and in vitro differentiation studies. In contrast, transplantation of undifferentiated iPSCs directly into the seminiferous tubules of germ cell-depleted immunodeficient mice revealed divergent fates of iPSCs produced with different factors. Transplantation resulted in morphologically and immunohistochemically recognizable germ cells in vivo, particularly in the case of OSKMV cells.

Eisenstein, A., S. H. Carroll, et al. "An Adenosine Receptor-Kruppel-like Factor 4 Protein Axis Inhibits Adipogenesis." J Biol Chem. 2014 Jul 25;289(30):21071-21081. Epub 2014 Jun 13.

Adipogenesis represents a key process in adipose tissue development and remodeling, including during obesity. Exploring the regulation of adipogenesis by extracellular ligands is fundamental to our understanding of this process. Adenosine, an extracellular nucleoside signaling molecule found in adipose tissue depots, acts on adenosine receptors. Here we report that, among these receptors, the A2b adenosine receptor (A2bAR) is highly expressed in adipocyte progenitors. Activation of the A2bAR potently inhibits differentiation of mouse stromal vascular cells into adipocytes, whereas A2bAR knockdown stimulates adipogenesis. The A2bAR inhibits differentiation through a novel signaling cascade involving sustained expression of Kruppel-like factor 4 (KLF4), a regulator of stem cell maintenance. Knockdown of KLF4 ablates the ability of the A2bAR to inhibit differentiation. A2bAR activation also inhibits adipogenesis in a human primary preadipocyte culture system. We analyzed the A2bAR-KLF4 axis in adipose tissue of obese subjects and, intriguingly, found a strong correlation between A2bAR and KLF4 expression in both subcutaneous and visceral human fat. Hence, our study implicates the A2bAR as a regulator of adipocyte differentiation and the A2bAR-KLF4 axis as a potentially significant modulator of adipose biology.

Fekete, N., A. Erle, et al. "Effect of High-Dose Irradiation on Human Bone-Marrow-Derived Mesenchymal Stromal Cells." Tissue Eng Part C Methods. 2014 Jul 22.

Cell therapy using multipotent mesenchymal stromal cells (MSCs) is of high interest in various indications. As the pleiotropic effects mediated by MSCs rely mostly on their unique secretory profile, long-term persistence of ex-vivo-expanded cells in the recipient may not always be desirable. Irradiation is a routine procedure in transfusion medicine to prevent long-term persistence of nucleated cells and could therefore also be applied to MSCs. We have exposed human bone-marrow-derived MSCs to 30 or 60 Gy of gamma-irradiation and assessed cell proliferation, clonogenicity, differentiation, cytokine levels in media supernatants, surface receptor profile, as well as expression of proto-oncogenes/cell cycle markers, self-renewal/stemness markers, and DNA damage/irradiation markers. Irradiated MSCs show a significant decrease in proliferation and colony-forming unit-fibroblasts. However, a subpopulation of surviving cells is able to differentiate, but is unable to form colonies after irradiation. Irradiated MSCs showed stable expression of CD73 and CD90 and absence of CD3, CD34, and CD45 during a 16-week follow-up period. We found increased vascular endothelial growth factor (VEGF) levels and a decrease of platelet-derived growth factor (PDGF)-AA and PDGF-AB/BB in culture media of nonirradiated cells. Irradiated MSCs showed an inverse pattern, that is, no increase of VEGF, and less consumption of PDGF-AA and PDGF-AB/BB. Interestingly, interleukin-6 (IL-6) levels increased during culture regardless of irradiation. Cells with lower sensitivity toward gamma-irradiation showed positive beta-galactosidase activity 10 days after irradiation. Gene expression of both irradiated and nonirradiated MSCs 13-16 weeks after irradiation with 60 Gy predominantly followed the same pattern; cell cycle regulators CDKN1A (p21) and CDKN2A (p16) were upregulated, indicating cell cycle arrest, whereas classical proto-oncogenes, respectively, and self-renewal/stemness markers MYC, TP53 (p53), and KLF4 were downregulated. In addition, DNA damage/irradiation markers ATM, ATR, BRCA1, CHEK1, CHEK2, MDC1, and TP53BP1 also mostly showed the same pattern of gene expression as high-dose gamma-irradiation. In conclusion, we demonstrated the existence of an MSC subpopulation with remarkable resistance to high-dose gamma-irradiation. Cells surviving irradiation retained their trilineage differentiation capacity and surface marker profile but changed their cytokine secretion profile and became prematurely senescent.

Finicelli, M., G. Benedetti, et al. "Expression of stemness genes in primary breast cancer tissues: the role of SOX2 as a prognostic marker for detection of early recurrence." Oncotarget. 2014 May 1.

The events leading to breast cancer (BC) progression or recurrence are not completely understood and new prognostic markers aiming at identifying high risk-patients and to develop suitable therapy are highly demanded. Experimental evidences found in cancer cells a deregulated expression of some genes involved in governance of stem cell properties and demonstrated a relationship between stemness genes overexpression and poorly differentiated BC subtypes. In the present study 140 primary invasive BC specimens were collected. The expression profiles of 13 genes belonging to the OCT3/SOX2/NANOG/KLF4 core circuitry by RT-PCR were analyzed and any correlation between their expression and the BC clinic-pathological features (CPfs) and prognosis was investigated. In our cohort (117 samples), NANOG, GDF3 and SOX2 significantly correlated with grade 2, Nodes negative status and higher KI67 proliferation index, respectively (p=0.019, p=0.029, p= 0.035). According to multivariate analysis, SOX2 expression resulted independently associated with increased risk of recurrence (HR= 2,99; p= p=0,004) as well as Nodes status (HR=2,44; p=0,009) and T-size >1 (HR=1,77; p=0,035) . Our study

provides further proof of the suitable use of stemness genes in BC management. Interestingly, a prognostic role of SOX2, which seems to be a suitable marker of early recurrence irrespective of other clinicopathological features.

Fujimura, T., S. Takahashi, et al. "Expression of androgen and estrogen signaling components and stem cell markers to predict cancer progression and cancer-specific survival in patients with metastatic prostate cancer." Clin Cancer Res. 2014 Sep 1;20(17):4625-35. doi: 10.1158/1078-0432.CCR-13-1105. Epub 2014 Jul 1.

PURPOSE: Genes of androgen and estrogen signaling cells and stem cell-like cells play crucial roles in prostate cancer. This study aimed to predict clinical failure by identifying these prostate cancer-related genes. EXPERIMENTAL DESIGN: We developed models to predict clinical failure using biopsy samples from a training set of 46 and an independent validation set of 30 patients with treatment-naive prostate cancer with bone metastasis. Cancerous and stromal tissues were separately collected by laser-captured microdissection. We analyzed the association between clinical failure and mRNA expression of the following genes androgen receptor (AR) and its related genes (APP, FOX family, TRIM 36, Oct1, and ACSL 3), stem cell-like molecules (Klf4, c-Myc, Oct 3/4, and Sox2), estrogen receptor (ER), Her2, PSA, and CRP. RESULTS: Logistic analyses to predict prostate-specific antigen (PSA) recurrence showed an area under the curve (AUC) of 1.0 in both sets for Sox2, Her2, and CRP expression in cancer cells, AR and ERalpha expression in stromal cells, and clinical parameters. We identified 10 prognostic factors for cancer-specific survival (CSS): Oct1, TRIM36, Sox2, and c-Myc expression in cancer cells; AR, Klf4, and ERalpha expression in stromal cells; and PSA, Gleason score, and extent of disease. On the basis of these factors, patients were divided into favorable-, intermediate-, and poor-risk groups according to the number of factors present. Five-year CSS rates for the 3 groups were 90%, 32%, and 12% in the training set and 75%, 48%, and 0% in the validation set, respectively. CONCLUSIONS: Expression levels of androgen- and estrogen signaling components and stem cell markers are powerful prognostic tools. Clin Cancer Res; 20(17); 4625-35. (c)2014 AACR.

Galiger, C., S. Kostin, et al. "Phenotypical and ultrastructural features of Oct4-positive cells in the adult mouse lung." J Cell Mol Med. 2014 Jul;18(7):1321-33. doi: 10.1111/jcmm.12295. Epub 2014 Jun 3.

Octamer binding trascription factor 4 (Oct4) is a transcription factor of POU family specifically expressed in embryonic stem cells (ESCs). A role for maintaining pluripotency and self-renewal of ESCs is assigned to Oct4 as a pluripotency marker. Oct4 can also be detected in adult stem cells such as bone marrow-derived mesenchymal stem cells. Several studies suggest a role for Oct4 in sustaining self-renewal capacity of adult stem cells. However, Oct4 gene ablation in adult stem cells revealed no abnormalities in tissue turnover or regenerative capacity. In the present study we have conspicuously found pulmonary Oct4-positive cells closely resembling the morphology of telocytes (TCs). These cells were found in the perivascular and peribronchial areas and their presence and location were confirmed by electron microscopy. Moreover, we have used Oct4-GFP transgenic mice which revealed a similar localization of the Oct4-GFP signal. We also found that Oct4 co-localized with several described TC markers such as vimentin, Sca-1, platelet-derived growth factor receptor-beta C-kit and VEGF. By flow cytometry analyses carried out with Oct4-GFP reporter mice, we described a population of EpCAM(neg) /CD45(neg) /Oct4-GFP(pos) that in culture displayed TC features. These results were supported by qRT-PCR with mRNA isolated from lungs by using laser capture microdissection. In addition, Oct4-positive cells were found to express Nanog and Klf4 mRNA. It is concluded for the first time that TCs in adult lung mouse tissue comprise Oct4-positive cells, which express pluripotency-related genes and represent therefore a population of adult stem cells which might contribute to lung regeneration.

Gallagher, C., C. Clarke, et al. "Comparative transcriptomic analysis of cultivated limbal epithelium and donor corneal tissue reveals altered wound healing gene expression." Invest Ophthalmol Vis Sci. 2014 Aug 14. pii: IOVS-14-14664. doi: 10.1167/iovs.14-14664.

Purpose: The improved surgical outcomes associated with cultivated amniotic membrane expanded limbal epithelial (AMLE) grafts compared to donor transplant methods have led to significant adoption of this technique for treatment of limbal stem cell deficiency. The molecular basis for the superior clinical performance of these grafts remains unclear. In this study, we compare the transcriptomes of AMLE and donor central corneal epithelium (CE) to investigate differential gene expression between these tissue types. Methods: The mRNA expression profiles of AMLE and CE were assayed using microarrays. Transcripts with a 1.5 fold change in either direction in addition to a Bonferroni adjusted p-value < 0.05 were considered to be differentially expressed. Expression changes detected by microarray profiling and important corneal-limbal markers were assessed using quantitative real-time PCR (qRT-PCR) and immunofluorescence staining. Results: 487 probesets (319 upregulated & 168 downregulated) were found to be differentially expressed between AMLE and CE. Enrichment analysis revealed significant overrepresentation of multiple biological processes (e.g. response to wounding, wound-healing and regulation of cell morphogenesis) within the differentially expressed gene-list. The expression of a number of genes that were upregulated (ABCG2, S100A9, ITGA5, TIMP2, FGF5, PDGFC, SEMA3A) and downregulated (KLF4, P63alpha) in AMLE were confirmed using qRT-PCR. Immunofluorescence confirmed that AMLE cultures were P63alpha, ABCG2, CK3, CK12 and E-cadherin (E-cad) positive. Conclusions: In this study we have shown that genes associated with wound healing processes are upregulated in AMLE. These gene expression changes may contribute to corneal restoration and the positive outcomes associated with transplantation.

Gardlik, R., A. Wagnerova, et al. "Effects of bacteriamediated reprogramming and antibiotic pretreatment on the course of colitis in mice." Mol Med Rep. 2014 Aug;10(2):983-8. doi: 10.3892/mmr.2014.2244. Epub 2014 May 16.

Since the original study by Takahashi and Yamanaka in 2006, there have been significant advances in the field of induced pluripotent stem cells. However, to the best of our knowledge, all of the studies published to date are based on ex vivo gene delivery and subsequent reimplantation of the cells. By contrast, in vivo reprogramming allows the direct administration of DNA encoding the reprogramming factors into the target tissue. In our previous study we demonstrated the beneficial effects of Salmonellamediated oral delivery of genes into colonic mucosa as a therapy for colitis. In the present study, the effect of the bacterial vector Salmonella typhimurium SL7207, carrying a plasmid encoding the reprogramming factors Sox2, Oct3/4 and Klf4, on colitis in mice was investigated. Therapeutic intervention, consisting of repeated gavaging following the induction of colitis, did not exhibit beneficial effects. However, preventive oral administration of the therapeutic bacterial strain resulted in improvements in weight loss, colon length and stool consistency. Recently it has been shown that antibiotic pretreatment may alleviate chemically induced colitis in mice. Therefore, in the present study it was investigated whether antibiotic pretreatment of mice was able to enhance colonization of the administered bacterial strain in the colon, and therefore improve therapeutic outcome. C57BL/6 mice were administered streptomycin and metronidazole for four days, prior to multiple oral administrations of therapeutic bacteria every other day. Following three gavages, mice were administered dextran sulfate sodium in their drinking water to induce colitis. Disease activity parameters, including stool consistency, weight loss and colon length, were

improved in the group receiving antibiotics and bacterial vectors. These results indicate that antibiotic pretreatment may enhance bacterial gene delivery into the colon. Furthermore, the anticipated in vivo reprogramming of colon cells appears to have a beneficial effect on the severity of colitis. These effects, however, still require further analyses.

Goyal, A., S. L. Chavez, et al. "Generation of human induced pluripotent stem cells using epigenetic regulators reveals a germ cell-like identity in partially reprogrammed colonies." PLoS One. 2013 Dec 12;8(12):e82838. doi: 10.1371/journal.pone.0082838. eCollection 2013.

Previous studies have shown that induced pluripotent stem cells (iPSCs) can be derived from fibroblasts by ectopic expression of four transcription factors, OCT4, SOX2, KLF4 and c-MYC using various methods. More recent studies have focused on identifying alternative approaches and factors that can be used to increase reprogramming efficiency of fibroblasts to pluripotency. Here, we use nucleofection, morpholino technologies and novel epigenetic factors, which were chosen based on their expression profile in human embryos, fibroblasts and undifferentiated/differentiated human embryonic stem cells (hESCs) and conventionally generated iPSCs, to reprogram human fibroblasts into iPSCs. By over expressing DNMT3B, AURKB, PRMT5 and/or silencing SETD7 in human fibroblasts with and without NANOG, hTERT and/or SV40 overexpression, we observed the formation of colonies resembling iPSCs that were positive for certain pluripotency markers, but exhibited minimal proliferation. More importantly, we also demonstrate that these partially-reprogrammed colonies express high levels of early to mid germ cell-specific genes regardless of the transfection approach, which suggests conversion to a germ cell-like identity is associated with early reprogramming. These findings may provide an additional means to evaluate human germ cell differentiation in vitro, particularly in the context of pluripotent stem cell-derived germ cell development, and contribute to our understanding of the epigenetic requirements of the reprogramming process.

Guzzo, R. M., V. Scanlon, et al. "Establishment of Human cell Type-Specific iPS cells with Enhanced Chondrogenic Potential." Stem Cell Rev. 2014 Jun 25.

The propensity of induced pluripotent stem (iPS) cells to differentiate into specific lineages may be influenced by a number of factors, including the selection of the somatic cell type used for reprogramming. Herein we report the generation of new iPS cells, which we derived from human articular chondrocytes and from cord blood mononucleocytes via lentiviral-mediated delivery of Oct4, Klf4, Sox2, and cMyc. Molecular, cytochemical, and cytogenic analyses confirmed the acquisition of hallmark features of pluripotency, as well as the retention of normal karyotypes following reprogramming of both the human articular chondrocytes (AC) and the cord blood (CB) cells. In vitro and in vivo functional analyses formally established the pluripotent differentiation capacity of all cell lines. Chondrogenic differentiation assays comparing iPS cells derived from AC, CB, and a well established dermal fibroblast cell line (HDFa-Yk26) identified enhanced proteoglycan-rich matrix formation and cartilage-associated gene expression from AC-derived iPS cells. These findings suggest that the tissue of origin may impact the fate potential of iPS cells for differentiating into specialized cell types, such as chondrocytes. Thus, we generated new cellular tools for the identification of inherent features driving high chondrogenic potential of reprogrammed cells.

Hale, A. T., H. Tian, et al. "Endothelial Kruppel-like factor 4 regulates angiogenesis and the Notch signaling pathway." J Biol Chem. 2014 Apr 25;289(17):12016-28. doi: 10.1074/jbc.M113.530956. Epub 2014 Mar 5.

Regulation of endothelial cell biology by the Notch signaling pathway (Notch) is essential to vascular development, homeostasis, and sprouting angiogenesis. Although Notch determines cell fate and differentiation in a wide variety of cells, the molecular basis of upstream regulation of Notch remains poorly understood. Our group and others have implicated the Kruppel-like factor family of transcription factors as critical regulators of endothelial function. Here, we show that Kruppel-like factor 4 (KLF4) is a central regulator of sprouting angiogenesis via regulating Notch. Using a murine model in which KLF4 is overexpressed exclusively in the endothelium, we found that sustained expression of KLF4 promotes ineffective angiogenesis leading to diminished tumor growth independent of endothelial cell proliferation or cell cycling effects. These tumors feature increased vessel density yet are hypoperfused, leading to tumor hypoxia. Mechanistically, we show that KLF4 differentially regulates expression of Notch receptors, ligands, and target genes. We also demonstrate that KLF4 limits cleavage-mediated activation of Notch1. Finally, we rescue Notch target gene expression and the KLF4 sprouting angiogenesis phenotype by supplementation of DLL4 recombinant protein. Identification of this hitherto undiscovered role of KLF4 implicates this transcription factor as a critical regulator of Notch, tumor angiogenesis, and sprouting angiogenesis.

Hall, V. J. and P. Hyttel "Breaking down pluripotency in the porcine embryo reveals both a premature and reticent stem cell state in the inner cell mass and unique expression profiles of the naive and primed stem cell States." Stem Cells Dev. 2014 Sep 1;23(17):2030-45. doi: 10.1089/scd.2013.0502. Epub 2014 Jun 20.

To date, it has been difficult to establish bona fide porcine embryonic stem cells (pESC) and stable induced pluripotent stem cells. Reasons for this remain unclear, but they may depend on inappropriate culture conditions. This study reports the most insights to date on genes expressed in the pluripotent cells of the porcine embryo, namely the inner cell mass (ICM), the trophectoderm-covered epiblast (EPI), and the embryonic disc epiblast (ED). Specifically, we reveal that the early porcine ICM represents a premature state of pluripotency due to lack of translation of key pluripotent proteins, and the late ICM enters a transient, reticent pluripotent state which lacks expression of most genes associated with pluripotency. We describe a unique expression profile of the porcine EPI, reflecting the naive stem cell state, including expression of OCT4, NANOG, CRIPTO, and SSEA-1; weak expression of NrOB1 and REX1; but very limited expression of genes in classical pathways involved in regulating pluripotency. The porcine ED, reflecting the primed stem cell state, can be characterized by the expression of OCT4, NANOG, SOX2, KLF4, cMYC, REX1, CRIPTO, and KLF2. Further cell culture experiments using inhibitors against FGF, JAK/STAT, BMP, WNT, and NODAL pathways on cell cultures derived from day 5 and 10 embryos reveal the importance of FGF, JAK/STAT, and BMP signaling in maintaining cell proliferation of pESCs in vitro. Together, this article provides new insights into the regulation of pluripotency, revealing unique stem cell states in the different porcine stem cell populations derived from the early developing embryo.

Hayashi, K., H. Sasamura, et al. "KLF4-dependent epigenetic remodeling modulates podocyte phenotypes and attenuates proteinuria." J Clin Invest. 2014 Jun 2;124(6):2523-37. doi: 10.1172/JCI69557. Epub 2014 May 8.

The transcription factor Kruppel-like factor 4 (KLF4) has the ability, along with other factors, to reprogram somatic cells into induced pluripotent stem (iPS) cells. Here, we determined that KLF4 is expressed in kidney glomerular podocytes and is decreased in both animal models and humans exhibiting a proteinuric. Transient restoration of KLF4 expression in podocytes of diseased glomeruli in vivo, either by gene transfer or transgenic expression, resulted in a sustained increase in nephrin expression and a decrease in albuminuria. In mice harboring podocyte-specific deletion of Klf4, adriamycin-induced proteinuria was substantially exacerbated, although these animals displayed minimal phenotypical changes

prior to adriamycin administration. KLF4 overexpression in cultured human podocytes increased expression of nephrin and other epithelial markers and reduced mesenchymal gene expression. DNA methylation profiling and bisulfite genomic sequencing revealed that KLF4 expression reduced methylation at the nephrin promoter and the promoters of other epithelial markers; however, methylation was increased at the promoters of genes encoding mesenchymal markers, suggesting selective epigenetic regulation of podocyte gene expression. Together, these results suggest that KLF4 epigenetically modulates podocyte phenotype and function and that the podocyte epigenome can be targeted for direct intervention and reduction of proteinuria.

Henderson, G. R., S. R. Brahmasani, et al. "Candidate gene expression patterns in rabbit preimplantation embryos developed in vivo and in vitro." J Assist Reprod Genet. 2014 Jul;31(7):899-911. doi: 10.1007/s10815-014-0233-0. Epub 2014 Apr 24.

PURPOSE: The levels and timing of expression of genes like BCLXL, HDAC1 and pluripotency marker genes namely, OCT4, SOX2, NANOG and KLF4 are known to influence preimplantation embryo development. Despite this information, precise understanding of their influence during preimplantation embryo development is lacking. The present study attempts to compare the expression of these genes in the in vivo and in vitro developed preimplantation embryos. METHODS: The in vivo and in vitro developed rabbit embryos collected at distinct developmental stages namely, pronuclear, 2 cell, 4 cell, 8 cell, 16 cell, Morula and blastocyst were compared at the transcriptional and translational levels using Real Time PCR and immunocytochemical studies respectively. RESULTS: The study establishes the altered levels of candidate genes at the transcriptional level and translational level with reference to the zygotic genome activation (ZGA) phase of embryo development in the in vivo and in vitro developed embryos. The expression of OCT4, KLF4, NANOG and SOX2 genes were higher in the in vitro developed embryos whereas and HDAC1 was lower. BCLXL expression had its peak at ZGA in in vivo developed embryos. Protein expression of all the candidate genes was observed in the embryos. BCLXL, KLF4 and NANOG exhibited diffused localisation whereas HDAC1, OCT4, and SOX2 exhibited nuclear localisation. CONCLUSIONS: This study leads to conclude that BCLXL peak expression at the ZGA phase may be a requirement for embryo development. Further expression of all the candidate genes was influenced by ZGA phase of development at the transcript level, but not at the protein level.

Hewitt, S. C., L. Li, et al. "Novel DNA motif binding activity observed in vivo with an estrogen receptor alpha mutant mouse." Mol Endocrinol. 2014 Jun;28(6):899-911. doi: 10.1210/me.2014-1051. Epub 2014 Apr 8.

Estrogen receptor alpha (ERalpha) interacts with DNA directly or indirectly via other transcription factors, referred to as "tethering." Evidence for tethering is based on in vitro studies and a widely used "KIKO" mouse model containing mutations that prevent direct estrogen response element DNA- binding. KIKO mice are infertile, due in part to the inability of estradiol (E2) to induce uterine epithelial proliferation. To elucidate the molecular events that prevent KIKO uterine growth, regulation of the pro-proliferative E2 target gene Klf4 and of Klf15, a progesterone (P4) target gene that opposes the pro-proliferative activity of KLF4, was evaluated. Klf4 induction was impaired in KIKO uteri; however, Klf15 was induced by E2 rather than by P4. Whole uterine chromatin immunoprecipitation-sequencing revealed enrichment of KIKO ERalpha binding to hormone response elements (HREs) motifs. KIKO binding to HRE motifs was verified using reporter gene and DNA-binding assays. Because the KIKO ERalpha has HRE DNA-binding activity, we evaluated the "EAAE" ERalpha, which has more severe DNA-binding domain mutations, and demonstrated a lack of estrogen response element or HRE reporter gene induction or DNA-binding. The EAAE mouse has an ERalpha null-like phenotype, with impaired uterine growth and transcriptional activity. Our findings demonstrate that the KIKO mouse model, which has been used by numerous investigators, cannot be used to establish biological functions for ERalpha tethering, because KIKO ERalpha effectively stimulates transcription using HRE motifs. The EAAE-ERalpha DNA-binding domain mutant mouse demonstrates that ERalpha DNA-binding is crucial for biological and transcriptional processes in reproductive tissues and that ERalpha tethering may not contribute to estrogen responsiveness in vivo.

Higuchi, S., T. M. Watanabe, et al. "Culturing of mouse and human cells on soft substrates promote the expression of stem cell markers." J Biosci Bioeng. 2014 Jun;117(6):749-55. doi: 10.1016/j.jbiosc.2013.11.011. Epub 2013 Dec 17.

Substrate elasticity is a potent regulator of the cell state. Soft substrates have been shown to promote the homogeneous self-renewal of mouse embryonic stem cells through the down-regulation of cell-matrix tractions. We therefore investigated whether soft substrates promote the reprogramming of somatic cells into induced pluripotent stem (iPS) cells. After retroviral infection with five factors, Oct3/4, Klf4, Sox2, Lin28 and Nanog, mouse embryonic fibroblasts (MEFs) were cultured on several artificial substrates of varying elasticity and examined for the expression of pluripotency genes. When MEFs were cultured on soft (<0.1 kPa) polyacrylamide gels coated with gelatin, the expressions of Nanog and Oct3/4 genes were higher than in cells cultured on rigid plastic dishes (approximately 10(6) kPa). The same result was obtained at higher elasticity (0.5 kPa) for adult human dermal fibroblasts (HDFa). We also examined whether reprogramming could be enhanced on soft substrates without exogenous gene introduction, finding that cells cultured on soft substrates in the presence of chemicals known to promote cell reprogramming exhibited up-regulated stem cell markers. These results suggest that controlling the substrate stiffness can enhance the initiation of cell reprogramming, which may lead to effective and reproducible iPS cell production.

Hirota, N., S. McCuaig, et al. "Serotonin augments smooth muscle differentiation of bone marrow stromal cells." Stem Cell Res. 2014 May;12(3):599-609. doi: 10.1016/j.scr.2014.02.003. Epub 2014 Feb 13.

Bone marrow stromal cells (BMSCs) contain a subset of multipotent stem cells. Here, we demonstrate that serotonin, a biogenic amine released by platelets and mast cells, can induce the smooth muscle differentiation of BMSCs. Brown Norway rat BMSCs stimulated with serotonin had increased expression of the smooth muscle markers smooth muscle myosin heavy chain (MHC) and alpha actin (alpha-SMA) by qPCR and Western blot, indicating smooth muscle differentiation. This was accompanied by a concomitant down-regulation of the microRNA miR-25-5p, which was found to negatively regulate smooth muscle differentiation. Serotonin upregulated serum response factor (SRF) and myocardin, transcription factors known to induce contractile protein expression in smooth muscle cells, while it down-regulated Elk1 and Kruppel-like factor 4 (KLF4), known to induce proliferation. Serotonin increased SRF binding to promoter regions of the MHC and alpha-SMA genes, assessed by chromatin immunoprecipitation assay. Induction of smooth muscle differentiation by serotonin was blocked by the knock-down of SRF and myocardin. Transforming growth factor (TGF)-beta1 was constitutively expressed by BMSCs and serotonin triggered its release. Inhibition of miR-25-5p augmented TGF-beta1 expression, however the differentiation of BMSCs was not mediated by TGF-beta1. These findings demonstrate that serotonin promotes a smooth muscle-like phenotype in BMSCs by altering the balance of SRF, myocardin, Elk1 and KLF4 and miR-25-5p is involved in modulating this balance. Therefore, serotonin potentially contributes to the pathogenesis of diseases characterized by tissue remodeling with increased smooth muscle mass.

Ichida, J. K., C. W. J. T, et al. "Notch inhibition allows oncogene-independent generation of iPS cells." Nat Chem Biol. 2014 Aug;10(8):632-9. doi: 10.1038/nchembio.1552. Epub 2014 Jun 22.

The reprogramming of somatic cells to pluripotency using defined transcription factors holds great promise for biomedicine. However, human reprogramming remains inefficient and relies either on the use of the potentially dangerous oncogenes KLF4 and CMYC or the genetic inhibition of the tumor suppressor gene p53. We hypothesized that inhibition of signal transduction pathways that promote differentiation of the target somatic cells during development might relieve the requirement for non-core pluripotency factors during induced pluripotent stem cell (iPSC) reprogramming. Here, we show that inhibition of Notch greatly improves the efficiency of iPSC generation from mouse and human keratinocytes by suppressing p21 in a p53-independent manner and thereby enriching for undifferentiated cells capable of long-term self-renewal. Pharmacological inhibition of Notch enabled routine production of human iPSCs without KLF4 and CMYC while leaving p53 activity intact. Thus, restricting the development of somatic cells by altering intercellular communication enables the production of safer human iPSCs.

Imbernon, M., E. Sanchez-Rebordelo, et al. "Hypothalamic KLF4 mediates leptin's effects on food intake via AgRP." Mol Metab. 2014 Apr 15;3(4):441-51. doi: 10.1016/j.molmet.2014.04.001. eCollection 2014 Jul.

Kruppel-like factor 4 (KLF4) is a zinc-finger-type transcription factor expressed in a range of tissues that plays multiple functions. We report that hypothalamic KLF4 represents a new transcription factor specifically modulating agouti-related protein (AgRP) expression in vivo. Hypothalamic KLF4 colocalizes with AgRP neurons and is modulated by nutritional status and leptin. Over-expression of KLF4 in the hypothalamic arcuate nucleus (ARC) induces food intake and increases body weight through the specific stimulation of AgRP, as well as blunting leptin sensitivity in lean rats independent of forkhead box protein 01 (FoxO1). Down-regulation of KLF4 in the ARC inhibits fasting-induced food intake in both lean and diet-induced obese (DIO) rats. Silencing KLF4, however, does not, on its own, enhance peripheral leptin sensitivity in DIO rats.

Isono, K., H. Jono, et al. "Generation of familial amyloidotic polyneuropathy-specific induced pluripotent stem cells." Stem Cell Res. 2014 Mar;12(2):574-83. doi: 10.1016/j.scr.2014.01.004. Epub 2014 Jan 27.

Familial amyloidotic polyneuropathy (FAP) is a hereditary amyloidosis induced by amyloidogenic transthyretin (ATTR). Because most transthyretin (TTR) in serum is synthesized by the liver, liver transplantation (LT) is today the only treatment available to halt the progression of FAP, even though LT is associated with several problems. Despite the urgent need to develop alternatives to LT, the detailed pathogenesis of FAP is still unknown; also, no model fully represents the relevant processes in patients with FAP. The induction of induced pluripotent stem (iPS) cells has allowed development of pluripotent cells specific for patients and has led to useful models of human diseases. Because of the need for a tool to elucidate the molecular pathogenesis of FAP, in this study we sought to establish heterozygous ATTR mutant iPS cells, and were successful, by using a Sendai virus vector mixture containing four transcription factors (Oct3/4, Sox2, Klf4, and c-Myc) to reprogram dermal fibroblasts derived from FAP patients. Moreover, FAP-specific iPS cells had the potential to differentiate into hepatocyte-like cells and indeed expressed ATTR. FAP-specific iPS cells demonstrated the possibility of serving as a pathological tool that will contribute to understanding the pathogenesis of FAP and development of FAP treatments.

Ji, Q., A. L. Fischer, et al. "Engineered zinc-finger transcription factors activate OCT4 (POU5F1), SOX2, KLF4, c-MYC (MYC) and miR302/367." Nucleic Acids Res. 2014;42(10):6158-67. doi: 10.1093/nar/gku243. Epub 2014 May 3.

Artificial transcription factors are powerful tools for regulating gene expression. Here we report results with engineered zinc-finger transcription factors (ZF-TFs) targeting four protein-coding genes, OCT4, SOX2, KLF4 and c-MYC, and one noncoding ribonucleic acid (RNA) gene, the microRNA (miRNA) miR302/367 cluster. We designed over 300 ZF-TFs whose targets lie within 1 kb of the transcriptional start sites (TSSs), screened them for increased messenger RNA or miRNA levels in transfected cells, and identified potent ZF-TF activators for each gene. Furthermore, we demonstrate that selected ZF-TFs function with alternative activation domains and in multiple cell lines. For OCT4, we expanded the target range to -2.5 kb and +500 bp relative to the TSS and identified additional active ZF-TFs, including three highly active ZF-TFs targeting distal enhancer, proximal enhancer and downstream from the proximal promoter. Chromatin immunoprecipitation (FLAG-ChIP) results indicate that several inactive ZF-TFs targeting within the same regulatory region bind as well as the most active ZF-TFs, suggesting that efficient binding within one of these regulatory regions may be necessary but not sufficient for activation. These results further our understanding of ZF-TF design principles and corroborate the use of ZF-TFs targeting enhancers and downstream from the TSS for transcriptional activation.

Kaul, D., S. Sharma, et al. "Arsenic programmes cellular genomic-immunity through miR-2909 RNomics." Gene. 2014 Feb 25;536(2):326-31. doi: 10.1016/j.gene.2013.12.004. Epub 2013 Dec 18.

It is widely recognized that human cells are equipped with innate antiviral-RNA armour involving the production of type I interferons and APOBEC3G (apolipoprotein B mRNA-editing, enzyme-catalytic, polypeptide-like 3G) gene-product. Although arsenic has been shown to have paradoxical effect on one arm of this armour involving APOBEC3G, the exact molecular mechanism of its action in this regard is far from clear. The present study, addressed to explore as to how arsenic programmes this innate antiviral-RNA cellular-sensing pathway, clearly revealed that arsenic programmes this innate cellular antiviral genomic response through its inherent capacity to initiate cellular miR-2909 RNomics pathway, involving not only the modulation of APOBEC3G gene but also KLF4 (Kruppel-like factor 4) dependent regulation of gene coding for IKBKepsilon (Inhibitor of nuclear factor kappa-B kinase subunit epsilon) which in turn modulates RIG-I (retinoic acid-inducible gene 1) pathway responsible for the production of IFNbeta (interferon beta) through restriction of CYLD (Cylindromatosis) deubiqutinating activity. This restricted inhibitory enzyme activity of CYLD upon NFkB (nuclear factor kappa-light-chain-enhancer of activated B cells) also ensures sustained expression of miR-2909. Our results for the first time show that cellular miR-2909 RNomics may constitute an innate genomic armour to promote as well as restrict retroviral infection.

Kidder, B. L. "Generation of induced pluripotent stem cells using chemical inhibition and three transcription factors." Methods Mol Biol. 2014;1150:227-36. doi: 10.1007/978-1-4939-0512-6_15.

Generation of induced pluripotent stem (iPS) cells from differentiated cells has traditionally been performed by overexpressing four transcription factors: Oct4, Sox2, Klf4, and c-Myc. However, inclusion of c-Myc in the reprogramming cocktail can lead to expansion of transformed cells that are not fully reprogrammed, and studies have demonstrated that c-Myc reactivation increases tumorigenicity in chimeras and progeny mice. Moreover, chemical inhibition of Wnt signaling has been shown to enhance reprogramming efficiency. Here, we describe a modified protocol for generating iPS cells from murine fibroblasts using chemical inhibition and overexpression of three transcription factors. Using this protocol, we observed robust conversion to iPS

cells while maintaining minimal contamination of partially reprogrammed transformed colonies.

Kimura, T., Y. Kaga, et al. "Pluripotent stem cells derived from mouse primordial germ cells by small molecule compounds." Stem Cells. 2014 Sep 3. doi: 10.1002/stem.1838.

Primordial germ cells (PGCs) can give rise to pluripotent stem cells known as embryonic germ cells (EGCs) when cultured with basic fibroblast growth factor (bFGF), stem cell factor (SCF), and leukemia inhibitory factor (LIF). Somatic cells can give rise to induced pluripotent stem cells (iPSCs) by introduction of the reprogramming transcription factors Oct4, Sox2, and Klf4. The effects of Sox2 and Klf4 on somatic cell reprogramming can be reproduced using the small molecule compounds, transforming growth factor-beta receptor (TGFbetaR) inhibitor and Kempaullone, respectively. Here we examined the effects of TGFbetaR inhibitor and Kempaullone on EGC derivation from PGCs. Treatment of PGCs with TGFbetaR inhibitor and/or Kempaullone generated pluripotent stem cells under standard embryonic stem cell (ESC) culture conditions without bFGF and SCF, which we termed induced EGCs (iEGCs). The derivation efficiency of iEGCs was dependent on the differentiation stage and sex. DNA methylation levels of imprinted genes in iEGCs were reduced, with the exception of the H19 gene. The promoters of genes involved in germline development were generally hypomethylated in PGCs, but three germline genes showed comparable DNA methylation levels among iEGs, ESCs, and iPSCs. These results show that PGCs can be reprogrammed into pluripotent state using small molecule compounds, and that DNA methylation of these germline genes is not maintained in iEGCs. Stem Cells 2014.

Kinikoglu, B., Y. Kong, et al. "Characterization of cultured multipotent zebrafish neural crest cells." Exp Biol Med (Maywood). 2014 Feb;239(2):159-68. doi: 10.1177/1535370213513997. Epub 2013 Dec 10.

The neural crest is a unique cell population associated with vertebrate evolution. Neural crest cells (NCCs) are characterized by their multipotent and migratory potentials. While zebrafish is a powerful genetic model organism, the isolation and culture of zebrafish NCCs would provide a useful adjunct to fully interrogate the genetic networks that regulate NCC development. Here we report for the first time the isolation, in vitro culture, and characterization of NCCs from zebrafish embryos. NCCs were isolated from transgenic sox10:egfp embryos using fluorescence activated cell sorting and cultured in complex culture medium without feeder layers. NCC multilineage differentiation was determined by immunocytochemistry and real-time qPCR, cell migration was assessed by wound healing assay, and the proliferation index was calculated by immunostaining against the mitosis marker phospho-histone H3. Cultured NCCs expressed major neural crest lineage markers such as sox10, sox9a, hnk1, p75, dlx2a, and pax3, and the pluripotency markers c-myc and klf4. We showed that the cultured NCCs can be differentiated into multiple neural crest lineages, contributing to neurons, glial cells, smooth muscle cells, melanocytes, and chondrocytes. We applied the NCC in vitro model to study the effect of retinoic acid on NCC development. We showed that retinoic acid had a profound effect on NCC morphology and differentiation, significantly inhibited proliferation and enhanced cell migration. The availability of high numbers of NCCs and reproducible functional assays offers new opportunities for mechanistic studies of neural crest development, in genetic and chemical biology applications.

Luz-Madrigal, A., E. Grajales-Esquivel, et al. "Reprogramming of the chick retinal pigmented epithelium after retinal injury." BMC Biol. 2014 Apr 17;12:28. doi: 10.1186/1741-7007-12-28.

BACKGROUND: One of the promises in regenerative medicine is to regenerate or replace damaged tissues. The embryonic chick can regenerate its retina by transdifferentiation of the retinal pigmented epithelium (RPE) and by activation of stem/progenitor cells present in the ciliary margin. These two ways of regeneration occur concomitantly when an external source of fibroblast growth factor 2 (FGF2) is present after injury (retinectomy). During the process of transdifferentiation, the RPE loses its pigmentation and is reprogrammed to become neuroepithelium, which differentiates to reconstitute the different cell types of the neural retina. Somatic mammalian cells can be reprogrammed to become induced pluripotent stem cells by ectopic expression of pluripotency-inducing factors such as Oct4, Sox2, Klf4, c-Myc and in some cases Nanog and Lin-28. However, there is limited information concerning the expression of these factors during natural regenerative processes. Organisms that are able to regenerate their organs could share similar mechanisms and factors with the reprogramming process of somatic cells. Herein, we investigate the expression of pluripotency-inducing factors in the RPE after retinectomy (injury) and during transdifferentiation in the presence of FGF2. RESULTS: We present evidence that upon injury, the quiescent (p27(Kip1)+/BrdU-) RPE cells transiently dedifferentiate and express sox2, c-myc and klf4 along with eye field transcriptional factors and display a differential up-regulation of alternative splice variants of pax6. However, this transient process of dedifferentiation is not sustained unless FGF2 is present. We have identified lin-28 as a downstream target of FGF2 during the process of retina regeneration. Moreover, we show that overexpression of lin-28 after retinectomy was sufficient to induce transdifferentiation of the RPE in the absence of FGF2. CONCLUSION: These findings delineate in detail the molecular changes that take place in the RPE during the process of transdifferentiation in the embryonic chick, and specifically identify Lin-28 as an important factor in this process. We propose a novel model in which injury signals initiate RPE dedifferentiation, while FGF2 up-regulates Lin-28, allowing for RPE transdifferentiation to proceed.

Ma, J., P. Wang, et al. "Kruppel-like factor 4 regulates blood-tumor barrier permeability via ZO-1, occludin and claudin-5." J Cell Physiol. 2014 Jul;229(7):916-26. doi: 10.1002/jcp.24523.

Blood-tumor barrier (BTB) constitutes an efficient organization of tight junctions which significantly reduce permeability for chemotherapy drugs. Kruppel-like factor 4 (KLF4), a member of the Kruppel-like family, has been documented in endothelial cells and may serve as an essential regulator of endothelial barrier function. However, our knowledge about the expression and function of KLF4 in the endothelial cells of BTB still remains unclear. In this study, we sought to investigate the role of KLF4 in regulation of BTB function as well as the potential molecular mechanisms. Quantitative RT-PCR, Western blot, and immunofluorescence assays demonstrated that KLF4 was down-regulated in the glioma endothelial cells (GECs) which were obtained through endothelial cells co-cultured with glioma cells. Short hairpin RNA targeting KLF4 impaired the integrity of BTB detected by trans-endothelial electric resistance assay, and meanwhile reduced the expression of ZO-1, occludin and claudin-5, demonstrated by quantitative RT-PCR, Western blot, and immunofluorescence assays. Depletion of KLF4 increased BTB permeability to small molecules detected by permeability assays. Furthermore, luciferase assays and chromatin immunoprecipitation assays showed that KLF4 up-regulated the promoter activities and interacted with "CACCC" DNA sequence presented in the promoters of ZO-1, occludin, and claudin-5. GATA-1, GATA-6, Sp1, and Sp3 factors participated in KLF4 regulation of promoter activities through binding to the promoters of tight junctions related proteins. Collectively, our results indicated that KLF4 is a key transcriptional regulator of BTB function by regulating expressions of tight junction related proteins, which would draw growing attention to KLF4 as a potential target for glioma therapy.

Ma, K., G. Song, et al. "miRNAs promote generation of porcine-induced pluripotent stem cells." Mol Cell Biochem. 2014

Apr;389(1-2):209-18. doi: 10.1007/s11010-013-1942-x. Epub 2014 Jan 24.

The pigs have similarities of organ size, immunology and physiology with humans. Porcine-induced pluripotent stem cells (piPSCs) have great potential application in regenerative medicine. Here, we established piPSCs induced from porcine fetal fibroblasts by the retroviral overexpression of Oct4, Sox2, Klf4, and c-Myc. The piPSCs not only express pluripotent markers but also have the capacity for differentiation in vivo and in vitro, including EB and teratoma formation. We supplemented microRNAs during the induction process because miR-302a, miR-302b, and miR-200c have been reported to be highly expressed in human and mouse embryonic stem cells and in iPSCs. In this study, we found that the overexpression of miR-302a, miR-302b, and miR-200c effectively improved the reprogramming efficiency and reduced the induction time for piPSCs in the OSKM and OSK induction systems. Due to the similar induction efficiency of 4F-induced piPSCs or of three factors combined with miR-302a, miR-302b, and miR-200c (3F-miRNA-induced piPSCs), we recommend the addition of miRNAs instead of c-Myc to reduce the tumorigenicity of piPSCs.

Ma, O., S. Hong, et al. "Granulopoiesis requires increased C/EBPalpha compared to monopoiesis, correlated with elevated Cebpa in immature G-CSF receptor versus M-CSF receptor expressing cells." PLoS One. 2014 Apr 21;9(4):e95784. doi: 10.1371/journal.pone.0095784. eCollection 2014.

C/EBPalpha is required for the formation of granulocyte-monocyte progenitors; however, its role in subsequent myeloid lineage specification remains uncertain. Transduction of murine marrow with either of two Cebpa shRNAs markedly increases monocyte and reduces granulocyte colonies in methylcellulose or the monocyte to neutrophil ratio in liquid culture. Similar findings were found after marrow shRNA transduction and transplantation and with CEBPA knockdown in human marrow CD34+ cells. These results apparently reflect altered myeloid lineage specification, as similar knockdown allowed nearly complete 32Dcl3 granulocytic maturation. Cebpa knockdown also generated lineage-negative blasts with increased colony replating capacity but unchanged cell cycle parameters, likely reflecting complete differentiation block. The shRNA having the greatest effect on lineage skewing reduced Cebpa 3-fold in differentiating cells but 6-fold in accumulating blasts. Indicating that Cebpa is the relevant shRNA target, shRNA-resistant C/EBPalpha-ER rescued marrow myelopoiesis. Cebpa knockdown in murine marrow cells also increased in vitro erythropoiesis, perhaps reflecting 1.6-fold reduction in PU.1 leading to GATA-1 derepression. Global gene expression analysis of lineage-negative blasts that accumulate after Cebpa knockdown demonstrated reduction in Cebpe and Gfi1, known transcriptional regulators of granulopoiesis, and also reduced Ets1 and Klf5. Populations enriched for immature granulocyte or monocyte progenitor/precursors were isolated by sorting Lin-Sca-1-c-Kit+ cells into GCSFR+MCSFR- or GCSFR-MCSFR+ subsets. Cebpa, Cebpe, Gfi1, Ets1, and Klf5 RNAs were increased in the c-Kit+GCSFR+ and Klf4 and Irf8 in the c-Kit+MCSFR+ populations, with PU.1 levels similar in both. In summary, higher levels of C/EBPalpha are required for granulocyte and lower levels for monocyte lineage specification, and this myeloid bifurcation may be facilitated by increased Cebpa gene expression in granulocyte compared with monocyte progenitors.

Madonna, R., Y. J. Geng, et al. "High glucose-induced hyperosmolarity impacts proliferation, cytoskeleton remodeling and migration of human induced pluripotent stem cells via aquaporin-1." Biochim Biophys Acta. 2014 Aug 7. pii: S0925-4439(14)00251-8. doi: 10.1016/j.bbadis.2014.07.030.

Background and objective: Hyperglycemia leads to adaptive cell responses in part due to hyperosmolarity. In endothelial and epithelial cells, hyperosmolarity induces aquaporin-1 (AQP1) which plays a role in cytoskeletal remodeling, cell proliferation and migration. Whether such impairments also occur in human induced pluripotent stem cells (iPS) is not known. We therefore investigated whether high glucose-induced hyperosmolarity impacts proliferation, migration, expression of pluripotency markers and actin skeleton remodeling in iPS cells in an AQP1-dependent manner. Methods and results: Human iPS cells were generated from skin fibroblasts by lentiviral transduction of four reprogramming factors (Oct4, Sox2, Klf4, c-Myc). After reprogramming, iPS cells were characterized by their adaptive responses to high glucose-induced hyperosmolarity by incubation with 5.5mmol/L glucose, high glucose (HG) at 30.5mM, or with the hyperosmolar control mannitol (HM). Exposure to either HG or HM increased the expression of AQP1. AQP1 co-immunoprecipitated with beta-catenin. HG and HM induced the expression of beta-catenin. Under these conditions, iPS cells showed increased ratios of F-actin to G-actin and formed increased tubing networks. Inhibition of AQP1 with small interfering RNA (siRNA) reverted the inducing effects of HG and HM. Conclusions: High glucose enhances human iPS cell proliferation and cytoskeletal remodeling due to hyperosmolarity-induced upregulation of AQP1.

Manukyan, M. and P. B. Singh "Epigenome rejuvenation: HP1beta mobility as a measure of pluripotent and senescent chromatin ground states." Sci Rep. 2014 Apr 25;4:4789. doi: 10.1038/srep04789.

We measured the dynamics of an essential epigenetic modifier, HP1beta, in human cells at different stages of differentiation using Fluorescence Recovery After Photobleaching (FRAP). We found that HP1beta mobility is similar in human embryonic stem cells (hES) and iPS cells where it is more mobile compared to fibroblasts; HP1beta is less mobile in senescent fibroblasts than in young (dividing) fibroblasts. Introduction of "reprogramming factors", Oct4, Sox2, Klf4, cMyc and Lin28, into senescent fibroblasts and measuring the changes in HP1beta mobility as reprogramming proceeds shows that the mobility of HP1beta in senescent cells increases and by day 9 is the same as that found in young fibroblasts. Thus the dynamics of a key epigenetic modifier can be rejuvenated without de-differentiation through an embryonic stage. Future work will test whether other aspects of cellular physiology that age can be so rejuvenated without de-differentiation.

Marinowic, D. R., M. F. Domingues, et al. "The expression of pluripotency genes and neuronal markers after neurodifferentiation in fibroblasts co-cultured with human umbilical cord blood mononuclear cells." In Vitro Cell Dev Biol Anim. 2014 Aug 19.

Human umbilical cord blood is an attractive source of stem cells; however, it has a heterogeneous cell population with few mesenchymal stem cells. Cell reprogramming induced by different methodologies can confer pluripotency to differentiated adult cells. The objective of this study was to evaluate the reprogramming of fibroblasts and their subsequent neural differentiation after co-culture with umbilical cord blood mononuclear cells. Cells were obtained from four human umbilical cords. The mononuclear cells were cultured for 7 d and subsequently co-cultured with mouse fibroblast NIH-3T3 cells for 6 d. The pluripotency of the cells was evaluated by RT-PCR using primers specific for pluripotency marker genes. The pluripotency was also confirmed by adipogenic and osteogenic differentiation. Neural differentiation of the reprogrammed cells was evaluated by immunofluorescence. All co-cultured cells showed adipogenic and osteogenic differentiation capacity. After co-cultivation, cells expressed the pluripotency gene KLF4. Statistically significant differences in cell area, diameter, optical density, and fractal dimension were observed by confocal microscopy in the neurally differentiated cells. Contact in the form of co-cultivation of fibroblasts with umbilical cord blood mononuclear fraction for 6 d promoted the reprogramming of these cells, allowing the later induction of neural differentiation.

Marthaler, A. G., U. Tiemann, et al. "Reprogramming to pluripotency through a somatic stem cell intermediate." PLoS One.

2013 Dec 27;8(12):e85138. doi: 10.1371/journal.pone.0085138. eCollection 2013.

Transcription factor-based reprogramming can lead to the successful switching of cell fates. We have recently reported that mouse embryonic fibroblasts (MEFs) can be directly reprogrammed into induced neural stem cells (iNSCs) after the forced expression of Brn4, Sox2, Klf4, and Myc. Here, we tested whether iNSCs could be further reprogrammed into induced pluripotent stem cells (iPSCs). The two factors Oct4 and Klf4 were sufficient to induce pluripotency in iNSCs. Immunocytochemistry and gene expression analysis showed that iNSC-derived iPSCs (iNdiPSCs) are similar to embryonic stem cells at the molecular level. In addition, iNdiPSCs could differentiate into cells of all three germ layers, both in vitro and in vivo, proving that iNdiPSCs are bona fide pluripotent cells. Furthermore, analysis of the global gene expression profile showed that iNdiPSCs, in contrast to iNSCs, do not retain any MEF transcriptional memory even at early passages after reprogramming. Overall, our results demonstrate that iNSCs can be reprogrammed to pluripotency and suggest that cell fate can be redirected numerous times. Importantly, our findings indicate that the induced pluripotent cell state may erase the donor-cell type epigenetic memory more efficiently than other induced somatic cell fates.

Martinez-Fernandez, A., T. J. Nelson, et al. "iPS Cell-Derived Cardiogenicity is Hindered by Sustained Integration of Reprogramming Transgenes." Circ Cardiovasc Genet. 2014 Jul 30. pii: CIRCGENETICS.113.000298.

BACKGROUND: -Nuclear reprogramming inculcates pluripotent capacity by which de novo tissue differentiation is enabled. Yet, introduction of ectopic reprogramming factors may desynchronize natural developmental schedules. This study aims to evaluate the impact of imposed transgene load on the cardiogenic competency of iPS cells. METHODS AND RESULTS: -Targeted inclusion and exclusion of reprogramming transgenes (c-MYC, KLF4, OCT4 and SOX2) was achieved using a drug-inducible and removable cassette according to the piggyBac transposon/transposase system. Pulsed transgene overexpression, prior to iPS cell differentiation, hindered cardiogenic outcomes. Delayed in counterparts with maintained integrated transgenes, transgene removal enabled proficient differentiation of iPS cells into functional cardiac tissue. Transgene-free iPS cells generated reproducible beating activity with robust expression of cardiac alpha-actinin, connexin 43, MLC2a, alpha/beta-MHC and troponin I. While operational excitation-contraction coupling was demonstrable in the presence or absence of transgenes, factor-free derivatives exhibited an expedited maturing phenotype with canonical responsiveness to adrenergic stimulation. CONCLUSIONS: -A disproportionate stemness load, caused by integrated transgenes, impacts the cardiogenic competency of iPS cells. Offload of transgenes in engineered iPS cells ensures integrity of cardiac developmental programs, underscoring the value of non-integrative nuclear reprogramming for derivation of competent cardiogenic regenerative biologics.

Matsumura, S., K. Higa, et al. "Characterization of mesenchymal progenitor cell populations from non-epithelial oral mucosa." Oral Dis. 2014 Sep 2. doi: 10.1111/odi.12288.

OBJECTIVES: The characteristics of cell populations extracted from oral mucosal non-epithelial tissues and their ability to differentiate were evaluated in vitro as a potential source of cells for mandibular and corneal regeneration. MATERIALS AND METHODS: Oral mucosal non-epithelial cells (OMNECs) were extracted from tissue samples, and were studied by flow cytometry and RT-PCR. Cells differentiating into osteoblasts, adipocytes, chondrocytes, neurocytes or keratocytes were characterized by RT-PCR and cell staining. RESULTS: OMNECs expressed CD44, CD90, CD105, CD166 and STRO-1 antigens, which are markers for mesenchymal stem cells. In addition, Oct3/4, c-Myc, Nanog, KLF4 and Rex, which are expressed by embryonic or pluripotent stem cells, were detected by RT-PCR. Expression of CD49d, CD56 and PDGFRalpha, proteins closely associated with the neural crest, was observed in OMNECs, as was expression of Twist1, Sox9, Snail1 and Snail2, which are early neural crest and neural markers. Specific differentiation markers were expressed in OMNECs after differentiation into osteoblasts, adipocytes, chondrocytes or keratocytes. CONCLUSIONS: Populations of OMNECs may contain both mesenchymal stem cells and neural crest origin cells, and are a potential cell source for autologous regeneration of mandibular or corneal stroma. This article is protected by copyright. All rights reserved.

Matsushita, N., H. Kobayashi, et al. "[Establishment of induced pluripotent stem cells from adipose tissue-derived stem cells for dendritic cell-based cancer vaccines]." Gan To Kagaku Ryoho. 2014 Apr;41(4):467-70.

Recently, studies on regenerative stem cell therapy are being encouraged, and efforts to generate dendritic cells, which play important roles in cancer immunotherapy, from stem cells are being made in the field of tumor immunology. Therapeutic acquisition of stem cells has important clinical applications. Studies on induced pluripotent stem(iPS)cells generated from somatic cells with pluripotent genes have advanced in recent years. Stem cells are reported to be found in adipose tissue (adipose-derived stem cells, ADSC). Our goal is to develop a new cancer vaccine by using dendritic cells generated from ADSC. In a preliminary study, we examined whether iPS cells can be generated from ADSC to serve as a source of dendritic cells.We introduced a plasmid with pluripotent genes(OCT3/4, KLF4, SOX2, L-MYC, LIN28, p53-shRNA)into an ADSC strain derived from adipose tissue by electroporation and subsequently cultured the cells for further examination. A colony sugges- tive of iPS cells from ADSC was observed. OCT3/4, KLF4, SOX2, L-MYC, and LIN28 mRNAs were expressed in the cultured cells, as confirmed by reverse transcriptase-polymerase chain reaction(RT-PCR). On the basis of these results, we confirmed that iPS cells were generated from ADSC. The method of inducing dendritic cells from iPS cells has already been reported, and the results of this study suggest that ADSC is a potential source of dendritic cells.

Mistry, D. S., Y. Chen, et al. "SNAI2 controls the undifferentiated state of human epidermal progenitor cells." Stem Cells. 2014 Aug 7. doi: 10.1002/stem.1809.

The transcription factor, SNAI2 is an inducer of the epithelial to mesenchymal transition (EMT) which mediates cell migration during development and tumor invasion. SNAI2 can also promote the generation of mammary epithelial stem cells from differentiated luminal cells when overexpressed. How SNAI2 regulates these critical and diverse functions is unclear. Here we show that the levels of SNAI2 expression are important for epidermal cell fate decisions. The expression of SNAI2 was found to be enriched in the basal layer of the interfollicular epidermis where progenitor cells reside and extinguished upon differentiation. Loss of SNAI2 resulted in premature differentiation whereas gain of SNAI2 expression inhibited differentiation. SNAI2 controls the differentiation status of epidermal progenitor cells by binding to and repressing the expression of differentiation genes with increased binding leading to further transcriptional silencing. Thus, the levels of SNAI2 binding to genomic targets determines the differentiation status of epithelial cells with increased levels triggering EMT and dedifferentiation, moderate (physiological) levels promoting epidermal progenitor function, and low levels leading to epidermal differentiation. Stem Cells 2014.

Morris, R., I. Sancho-Martinez, et al. "Mathematical approaches to modeling development and reprogramming." Proc Natl Acad Sci U S A. 2014 Apr 8;111(14):5076-82. doi: 10.1073/pnas.1317150111. Epub 2014 Mar 20.

Induced pluripotent stem cells (iPSCs) are created by the reprogramming of somatic cells via overexpression of certain

transcription factors, such as the originally described Yamanaka factors: Oct4, Sox2, Klf4, and c-Myc (OSKM). Here we discuss recent advancements in iPSC reprogramming and introduce mathematical approaches to help map the landscape between cell states during reprogramming. Our modelization indicates that OSKM expression diminishes and/or changes potential barriers between cell states and that epigenetic remodeling facilitate these transitions. From a practical perspective, the modeling approaches outlined here allow us to predict the time necessary to create a given number of iPSC colonies or the number of reprogrammed cells generated in a given time. Additional investigations will help to further refine modeling strategies, rendering them applicable toward the study of the development and stability of cancer cells or even other reprogramming processes such as lineage conversion. Ultimately, a quantitative understanding of cell state transitions might facilitate the establishment of regenerative medicine strategies and enhance the translation of reprogramming technologies into the clinic.

Muchkaeva, I. A., E. B. Dashinimaev, et al. "Generation of iPS Cells from Human Hair Follice Dermal Papilla Cells." Acta Naturae. 2014 Jan;6(1):45-53.

Dermal papilla (DP) cells are unique regional stem cells of the skin that induce formation of a hair follicle and its regeneration cycle. DP are multipotent stem cells; therefore we supposed that the efficiency of DPC reprogramming could exceed that of dermal fibroblasts reprogramming. We generated induced pluripotent stem cells from human DP cells using lentiviral transfection with Oct4, Sox2, Klf4, and c-Myc, and cultivation of cells both in a medium supplemented with valproic acid and at a physiological level of oxygen (5%). The efficiency of DP cells reprogramming was ~0.03%, while the efficiency of dermal fibroblast reprogramming under the same conditions was ~0.01%. Therefore, we demonstrated the suitability of DP cells as an alternative source of iPS cells.

Murtha, M., Z. Tokcaer-Keskin, et al. "FIREWACh: high-throughput functional detection of transcriptional regulatory modules in mammalian cells." Nat Methods. 2014 May;11(5):559-65. doi: 10.1038/nmeth.2885. Epub 2014 Mar 23.

Promoters and enhancers establish precise gene transcription patterns. The development of functional approaches for their identification in mammalian cells has been complicated by the size of these genomes. Here we report a high-throughput functional assay for directly identifying active promoter and enhancer elements called FIREWACh (Functional Identification of Regulatory Elements Within Accessible Chromatin), which we used to simultaneously assess over 80,000 DNA fragments derived from nucleosome-free regions within the chromatin of embryonic stem cells (ESCs) and identify 6,364 active regulatory elements. Many of these represent newly discovered ESC-specific enhancers, showing enriched binding-site motifs for ESC-specific transcription factors including SOX2, POU5F1 (OCT4) and KLF4. The application of FIREWACh to additional cultured cell types will facilitate functional annotation of the genome and expand our view of transcriptional network dynamics.

Naranjo Gomez, J. M., J. F. Bernal, et al. "Alterations in the expression of p53, KLF4, and p21 in neuroendocrine lung tumors." Arch Pathol Lab Med. 2014 Jul;138(7):936-42. doi: 10.5858/arpa.2013-0119-OA.

CONTEXT: Neuroendocrine lung neoplasms are a heterogeneous group of tumors with different clinical behavior and prognosis. OBJECTIVES: To evaluate the expression of p53, KLF4, and p21 in neuroendocrine lung neoplasms and to analyze the influence that expression has on the prognosis of those tumors. DESIGN: All neuroendocrine lung neoplasms (N = 109) resected in our institution were reviewed, with the collection of histologic slides and paraffin blocks of 47 typical carcinoids (43%), 9 atypical carcinoids (8%), 35 large cell neuroendocrine carcinomas (32%), and 18 small cell lung carcinomas (17%), as well as 10 tumorlets (100%). Four tissue microarrays were performed. Follow-up was assessed in all cases (119 of 119; 100%). RESULTS: p53 protein immunostaining results were negative in both the tumorlets and typical carcinoids and were overexpressed in 11% (1 of 9) of the atypical carcinoids and in 68% (36 of 53) of the carcinomas. KLF4 results were positive in all tumorlets (10 of 10; 100%), 32% (15 of 47) of the typical carcinoids, 44% (4 of 9) of the atypical carcinoids, and 62% (33 of 53) of the carcinomas. p21 expression did not differ among the groups. The lack of KLF4 and p21 expression was associated with an accumulation of aggressive features in typical carcinoids ($P = .04$ and $P = .004$, respectively, Fisher exact test). CONCLUSIONS: p53, KLF4, and p21 showed altered expression patterns in pulmonary neuroendocrine neoplasms. Lack of KLF4 and p21 expression was associated with accumulation of aggressive features in typical carcinoids.

Ni, A., M. J. Wu, et al. "Sphere Formation Permits Oct4 Reprogramming of Ciliary Body Epithelial Cells into Induced Pluripotent Stem Cells." Stem Cells Dev. 2014 Aug 18.

Somatic cells can be reprogrammed to induced pluripotent stem (iPS) cells by defined sets of transcription factors. We previously described reprogramming of monolayer-cultured adult mouse ciliary body epithelial (CE) cells by Oct4 and Klf4, but not with Oct4 alone. In this study, we report that Oct4 alone is sufficient to reprogram CE cells to iPS cells through sphere formation. Furthermore, we demonstrate that sphere formation induces a partial reprogramming state characterized by expression of retinal progenitor markers, upregulation of reprogramming transcription factors, such as Sall4 and Nanog, demethylation in the promoter regions of pluripotency associated genes, and mesenchymal to epithelial transition. The Oct4-iPS cells maintained normal karyotypes, expressed markers for pluripotent stem cells, and were capable of differentiating into derivatives of all three embryonic germ layers in vivo and in vitro. These findings suggest that sphere formation may render somatic cells more susceptible to reprogramming.

Ohnuki, M., K. Tanabe, et al. "Dynamic regulation of human endogenous retroviruses mediates factor-induced reprogramming and differentiation potential." Proc Natl Acad Sci U S A. 2014 Aug 26;111(34):12426-31. doi: 10.1073/pnas.1413299111. Epub 2014 Aug 5.

Pluripotency can be induced in somatic cells by overexpressing transcription factors, including POU class 5 homeobox 1 (OCT3/4), sex determining region Y-box 2 (SOX2), Kruppel-like factor 4 (KLF4), and myelocytomatosis oncogene (c-MYC). However, some induced pluripotent stem cells (iPSCs) exhibit defective differentiation and inappropriate maintenance of pluripotency features. Here we show that dynamic regulation of human endogenous retroviruses (HERVs) is important in the reprogramming process toward iPSCs, and in re-establishment of differentiation potential. During reprogramming, OCT3/4, SOX2, and KLF4 transiently hyperactivated LTR7s-the long-terminal repeats of HERV type-H (HERV-H)-to levels much higher than in embryonic stem cells by direct occupation of LTR7 sites genome-wide. Knocking down LTR7s or long intergenic non-protein coding RNA, regulator of reprogramming (lincRNA-RoR), a HERV-H-driven long noncoding RNA, early in reprogramming markedly reduced the efficiency of iPSC generation. KLF4 and LTR7 expression decreased to levels comparable with embryonic stem cells once reprogramming was complete, but failure to resuppress KLF4 and LTR7s resulted in defective differentiation. We also observed defective differentiation and LTR7 activation when iPSCs had forced expression of KLF4. However, when aberrantly expressed KLF4 or LTR7s were suppressed in defective iPSCs, normal differentiation was restored. Thus, a major mechanism by which OCT3/4, SOX2, and KLF4 promote human iPSC generation and reestablish potential for differentiation is by dynamically regulating HERV-H LTR7s.

Oktem, G., A. Bilir, et al. "Expression profiling of stem cell signaling alters with spheroid formation in CD133/CD44 prostate cancer stem cells." Oncol Lett. 2014 Jun;7(6):2103-2109. Epub 2014 Mar 24.

Cancer stem cells (CSC) isolated from multiple tumor types differentiate in vivo and in vitro when cultured in serum; however, the factors responsible for their differentiation have not yet been identified. The first aim of the present study was to identify CD133high/CD44high DU145 prostate CSCs and compare their profiles with non-CSCs as bulk counterparts of the population. Subsequently, the two populations continued to be three-dimensional multicellular spheroids. Differentiation was then investigated with stem cell-related genomic characteristics. Polymerase chain reaction array analyses of cell cycle regulation, embryonic and mesenchymal cell lineage-related markers, and telomerase reverse transcriptase (TERT) and Notch signaling were performed. Immunohistochemistry of CD117, Notch1, Jagged1, Delta1, Sox2, c-Myc, Oct4, KLF4, CD90 and SSEA1 were determined in CSC and non-CSC monolayer and spheroid subcultures. Significant gene alterations were observed in the CD133high/CD44high population when cultured as a monolayer and continued as spheroid. In this group, marked gene upregulation was determined in collagen type 9 alpha1, Islet1 and cyclin D2. Jagged1, Delta-like 3 and Notch1 were respectively upregulated genes in the Notch signaling pathway. According to immunoreactivity, the staining density of Jagged1, Sox2, Oct4 and Klf-4 increased significantly in CSC spheroids. Isolated CSCs alter their cellular characterization over the course of time and exhibit a differentiation profile while maintaining their former surface antigens at a level of transcription or translation. The current study suggested that this differentiation process may be a mechanism responsible for the malignant process and tumor growth.

Oktem, G., C. Sahin, et al. "Altered Stem Cell Receptor Activity in the Ovarian Surface Epithelium by Exogenous Zinc and/or Progesterone." Drug Res (Stuttg). 2014 Jul 3.

Background: Ovarian surface epithelium (OSE) has the characteristics of a stem cell and the potential for differentiation. Previous studies on this subject have succeeded in deriving oocytes from OSE stem cells, leading to the belief that OSE could be used for infertility treatment. Methods: Each rat (n=10) was subjected to zinc and/or progesterone injection for 5 days after conception. After a 6-day implantation period, ovarian tissues were removed and comprehensive immunohistochemical analysis of stem cell markers was conducted: Sox2, Klf4, Oct3/4, c-Myc, CD117, CD90, SSEA-1 and Notch pathway analysis; Notch1, Jagged1, and Delta1 in the OSE and ovarian stromal cells were evaluated after treatment with zinc, progesterone, or both. Results: Progesterone moderately affected Sox2 expression ($p<0.001$), while zinc application strongly affected Klf4 and Oct3/4 and immunoreactivity ($p<0.001$). CD90 immunoreactivity was decreased in the OSE and stroma of the progesterone group ($p=0.006$) compared with the zinc ($p = 0.244$) and zinc/progesterone groups ($p=0.910$). On the other hand, SSEA-1 showed moderate staining in the OSE and weak staining in stromal cells in animals treated with zinc ($p=0.727$), progesterone ($p=0.626$), and zinc/progesterone ($p=0.371$), with no differences compared with control. Zinc application affected Notch pathway immunoreactivity, with a significant increase in Notch1 ($p=0.0015$) and Jagged1 ($p<0.001$). Conclusions: The expression of putative stem cell markers in the OSE was verified and stem cell receptor activity was raised in the OSE and ovarian stromal cells by zinc and progesterone. Thus, this increased expression allows the therapeutic use of zinc and progesterone in ovary-related infertility and brings a different perspective to reproductive medicine.

Oshima, N., Y. Yamada, et al. "Induction of cancer stem cell properties in colon cancer cells by defined factors." PLoS One. 2014 Jul 9;9(7):e101735. doi: 10.1371/journal.pone.0101735. eCollection 2014.

Cancer stem cells (CSCs) are considered to be responsible for the dismal prognosis of cancer patients. However, little is known about the molecular mechanisms underlying the acquisition and maintenance of CSC properties in cancer cells because of their rarity in clinical samples. We herein induced CSC properties in cancer cells using defined factors. We retrovirally introduced a set of defined factors (OCT3/4, SOX2 and KLF4) into human colon cancer cells, followed by culture with conventional serum-containing medium, not human embryonic stem cell medium. We then evaluated the CSC properties in the cells. The colon cancer cells transduced with the three factors showed significantly enhanced CSC properties in terms of the marker gene expression, sphere formation, chemoresistance and tumorigenicity. We designated the cells with CSC properties induced by the factors, a subset of the transduced cells, as induced CSCs (iCSCs). Moreover, we established a novel technology to isolate and collect the iCSCs based on the differences in the degree of the dye-effluxing activity enhancement. The xenografts derived from our iCSCs were not teratomas. Notably, in contrast to the tumors from the parental cancer cells, the iCSC-based tumors mimicked actual human colon cancer tissues in terms of their immunohistological findings, which showed colonic lineage differentiation. In addition, we confirmed that the phenotypes of our iCSCs were reproducible in serial transplantation experiments. By introducing defined factors, we generated iCSCs with lineage specificity directly from cancer cells, not via an induced pluripotent stem cell state. The novel method enables us to obtain abundant materials of CSCs that not only have enhanced tumorigenicity, but also the ability to differentiate to recapitulate a specific type of cancer tissues. Our method can be of great value to fully understand CSCs and develop new therapies targeting CSCs.

Parchem, R. J., J. Ye, et al. "Two miRNA clusters reveal alternative paths in late-stage reprogramming." Cell Stem Cell. 2014 May 1;14(5):617-31. doi: 10.1016/j.stem.2014.01.021. Epub 2014 Mar 13.

Ectopic expression of specific factors such as Oct4, Sox2, and Klf4 (OSK) is sufficient to reprogram somatic cells into induced pluripotent stem cells (iPSCs). In this study, we examine the paths taken by cells during the reprogramming process by following the transcriptional activation of two pluripotent miRNA clusters (mir-290 and mir-302) in individual cells in vivo and in vitro with knockin reporters. During embryonic development and embryonic stem cell differentiation, all cells sequentially expressed mir-290 and mir-302. In contrast, during OSK-induced reprogramming, cells activated the miRNA loci in a stochastic, nonordered manner. However, the addition of Sall4 to the OSK cocktail led to a consistent reverse sequence of locus activation (mir-302 then mir-290) and increased reprogramming efficiency. These results demonstrate that cells can follow multiple paths during the late stages of reprogramming, and that the trajectory of any individual cell is strongly influenced by the combination of factors introduced.

Park, H., D. Kim, et al. "Increased genomic integrity of an improved protein-based mouse induced pluripotent stem cell method compared with current viral-induced strategies." Stem Cells Transl Med. 2014 May;3(5):599-609. doi: 10.5966/sctm.2013-0149. Epub 2014 Apr 24.

It has recently been shown that genomic integrity (with respect to copy number variants [CNVs]) is compromised in human induced pluripotent stem cells (iPSCs) generated by viral-based ectopic expression of specific transcription factors (e.g., Oct4, Sox2, Klf4, and c-Myc). However, it is unclear how different methods for iPSC generation compare with one another with respect to CNV formation. Because array-based methods remain the gold standard for detecting unbalanced structural variants (i.e., CNVs), we have used this approach to comprehensively identify CNVs in iPSC as a proxy for determining whether our modified protein-based method minimizes genomic instability compared with retro- and lentiviral

methods. In this study, we established an improved method for protein reprogramming by using partially purified reprogramming proteins, resulting in more efficient generation of iPSCs from C57/BL6J mouse hepatocytes than using protein extracts. We also developed a robust and unbiased 1 M custom array CGH platform to identify novel CNVs and previously described hot spots for CNV formation, allowing us to detect CNVs down to the size of 1.9 kb. The genomic integrity of these protein-based mouse iPSCs (p-miPSCs) was compared with miPSCs developed from viral-based strategies (i.e., retroviral: retro-miPSCs or lentiviral: lenti-miPSCs). We identified an increased CNV content in lenti-miPSCs and retro-miPSCs (29 approximately 53 CNVs) compared with p-miPSCs (9 approximately 10 CNVs), indicating that our improved protein-based reprogramming method maintains genomic integrity better than current viral reprogramming methods. Thus, our study, for the first time to our knowledge, demonstrates that reprogramming methods significantly influence the genomic integrity of resulting iPSCs.

Ramakrishna, S., K. S. Kim, et al. "Posttranslational modifications of defined embryonic reprogramming transcription factors." Cell Reprogram. 2014 Apr;16(2):108-20. doi: 10.1089/cell.2013.0077. Epub 2014 Feb 25.

The generation of induced pluripotent stem cells (iPSCs) from somatic cells by expressing ectopic reprogramming transcriptional factors such as Oct3/4, Sox2, Klf4, c-Myc, and Nanog is one of the cutting-edge discoveries in stem cell and cancer research. This discovery has raised several safety issues regarding the use of iPSC technology for human disease research. Tumorigenesis is the major obstacle observed for iPSC-mediated transplantation therapy. Recently, a new method to generate human iPSCs either by a chemical method or by direct delivery of reprogramming factors has become a promising approach for future customized cell therapy of human disorders. These reprogramming transcriptional factors play critical roles in diverse cellular functions such as transactivation, cellular proliferation, differentiation, apoptosis, and tumorigenesis. Posttranslational modifications (PTMs) (phosphorylation, ubiquitination, acetylation, sumoylation, and so on) of these proteins act as a regulatory signal to control protein activity, expression, and stability in a wide variety of cellular processes. We attempt to summarize the accumulated evidence to address the role of PTMs of Oct3/4, Sox2, Klf4, c-Myc, and Nanog in regulating their biological functions. This review allows us to understand the importance of PTMs and their application in developing an efficient and safe reprogramming method without cancer development for cell therapy. Finally, we discuss the importance of PTMs of reprogramming factors in tumor pathogenesis.

Ruetz, T. and K. Kaji Routes to induced pluripotent stem cells, Curr Opin Genet Dev. 2014 Sep 2;28C:38-42. doi: 10.1016/j.gde.2014.08.006.

The generation of induced pluripotent stem cells (iPSCs) with Oct4, Sox2, Klf4, c-Myc has been described as 'direct' reprogramming in contrast to reprogramming via nuclear transfer. Interestingly, recent studies have suggested that the conversion process itself includes transient up-regulation and down-regulation of hundreds of genes, making unique intermediate populations. In a sense, the process of 4 factor reprogramming is indirect. Like in vitro differentiation, iPSC generation efficiency and kinetics largely depend on the external environment, as well as the amount and stoichiometry of exogenously expressed reprogramming factors. However, accumulating evidence indicates that when reprogramming succeeds, the process is not random but progresses in an ordered, step-wise manner. In this review, we summarize current knowledge detailing how somatic cells reach a pluripotent state.

Saadeldin, I. M., S. J. Kim, et al. "Improvement of cloned embryos development by co-culturing with parthenotes: a possible role of exosomes/microvesicles for embryos paracrine communication." Cell Reprogram. 2014 Jun;16(3):223-34. doi: 10.1089/cell.2014.0003. Epub 2014 Apr 28.

It is well known that embryos cultured in a group can create a microenvironment through secretion of autocrine and paracrine factors that can support and improve the embryos' development when compared to the embryos cultured individually. In this study, we used a co-culture system for paracrine communication between different kinds of embryos. The results showed that co-culture of porcine parthenogenetic (PA) embryos significantly improved the in vitro development of cloned (nuclear transfer, NT) embryos. To reveal the possible mechanism of communication between the two groups, we isolated exosomes/microvesicles (EXs/MVs) from the PA embryos conditioned medium (PA-CM) through differential centrifugation and identified them through transmission electron microscope and immunoflourescence against exosomal/membrane marker CD9. Furthermore, these EXs/MVs were found to contain mRNA of pluripotency genes (Oct4, Sox2, Klf4, c-Myc, and Nanog), and the PKH67-labeled EXs/MVs could be internalized by the NT embryos. The current study demonstrates that cloned embryos' developmental competence can be improved through co-culturing with PA embryos and revealed, for the first time, that in vitro-produced embryos can secrete EXs/MVs as a possible communication tool within their microenvironment. Moreover, it provides a new paradigm for embryo-to-embryo communication in vitro.

Schott, J. W., D. Hoffmann, et al. "Improved retroviral episome transfer of transcription factors enables sustained cell fate modification." Gene Ther. 2014 Aug 7. doi: 10.1038/gt.2014.69.

Retroviral vectors are versatile gene transfer vehicles widely used in basic research and gene therapy. Mutation of retroviral integrase converts these vectors into transient, integration-deficient gene delivery vehicles associated with a high degree of biosafety. We explored the option to use integration-deficient retroviral vectors to achieve transient ectopic expression of transcription factors, which is considered an important tool for induced cell fate conversion. Stepwise optimization of the retroviral episome transfer as exemplified for the transcription factor Oct4 enabled to improve both expression magnitude and endurance. Long terminal repeat-driven gamma-retroviral vectors were identified as the most suitable vector architecture. Episomal expression was enhanced by epigenetic modifiers, and Oct4 activity was increased following fusion to a minimal transactivation motif of herpes simplex virus VP16. Based on kinetic analyses, we identified optimal time intervals for repeated vector administration and established prolonged expression windows of choice. Providing proof-of-concept, episomal transfer of Oct4 was potent to mediate conversion of human fibroblasts stably expressing Klf4, Sox2 and c-Myc into induced pluripotent stem cells, which were mainly free of residual Oct4 vector integration. This study provides evidence for suitability of retroviral episome transfer of transcription factors for cell fate conversion, allowing the generation of distinct patient- or disease-specific cell types.Gene Therapy advance online publication, 7 August 2014; doi:10.1038/gt.2014.69.

Schwarz, B. A., O. Bar-Nur, et al. "Nanog is dispensable for the generation of induced pluripotent stem cells." Curr Biol. 2014 Feb 3;24(3):347-50. doi: 10.1016/j.cub.2013.12.050. Epub 2014 Jan 23.

Cellular reprogramming from somatic cells to induced pluripotent stem cells (iPSCs) can be achieved through forced expression of the transcription factors Oct4, Klf4, Sox2, and c-Myc (OKSM) [1-4]. These factors, in combination with environmental cues, induce a stable intrinsic pluripotency network that confers indefinite self-renewal capacity on iPSCs. In addition to Oct4 and Sox2, the homeodomain-containing transcription factor Nanog is an integral part of the pluripotency network [5-11]. Although Nanog expression is not required for the maintenance of pluripotent stem cells, it has been reported to be essential for the establishment of both embryonic stem cells (ESCs) from blastocysts and iPSCs from

somatic cells [10, 12]. Here we revisit the role of Nanog in direct reprogramming. Surprisingly, we find that Nanog is dispensable for iPSC formation under optimized culture conditions. We further document that Nanog-deficient iPSCs are transcriptionally highly similar to wild-type iPSCs and support the generation of teratomas and chimeric mice. Lastly, we provide evidence that the presence of ascorbic acid in the culture media is critical for overcoming the previously observed reprogramming block of Nanog knockout cells.

Sharma, S. B., C. C. Lin, et al. "microRNAs-206 and -21 cooperate to promote RAS-ERK signaling by suppressing the translation of RASA1 and SPRED1." Mol Cell Biol. 2014 Sep 8. pii: MCB.00480-14.

Despite the low prevalence of activating point mutation of RAS or RAF genes, the RAS-ERK pathway is implicated in breast cancer pathogenesis. Indeed, in triple-negative breast cancer (TNBC) there is recurrent genetic alteration of pathway components. Using shRNA methods, we observed that the zinc finger transcription factor Kruppel-like factor 4 (KLF4) can promote RAS-ERK signaling in TNBC cells. Endogenous KLF4 bound to the promoter regions and promoted the expression of two microRNAs (miRs), miR-206 and miR-21 (miR-206/21). Antisense-mediated knockdown (anti-miR) revealed that miR-206/21 coordinately promote RAS-ERK signaling and the corresponding cell phenotypes by inhibiting translation of the pathway suppressors RASA1 and SPRED1. In TNBC cells, including cells with mutation of RAS, the suppression of either RASA1 or SPRED1 increased the levels of GTP-bound, wild-type RAS and activated ERK 1/2. Unlike the control cells, treatment of RASA1- or SPRED1-suppressed cells with anti-miR-206/21 had little or no impact on the level of activated ERK 1/2 or on cell proliferation, and failed to suppress tumor initiation. These results identify RASA1 and SPRED1 mRNAs as latent RAS-ERK pathway suppressors that can be upregulated in tumor cells by anti-miR treatment. Consequently, KLF4-regulated miRs are important for the maintenance of RAS-ERK pathway activity in TNBC cells.

Srivastava, R., R. Micanovic, et al. "An intricate network of conserved DNA upstream motifs and associated transcription factors regulate the expression of uromodulin gene." J Urol. 2014 Sep;192(3):981-9. doi: 10.1016/j.juro.2014.02.095. Epub 2014 Mar 1.

PURPOSE: Uromodulin is a kidney specific glycoprotein whose expression can modulate kidney homeostasis. However, the set of sequence specific transcription factors that regulate the uromodulin gene UMOD and their upstream binding locations are not well characterized. We built a high resolution map of its transcriptional regulation. MATERIALS AND METHODS: We applied in silico phylogenetic footprinting on the upstream regulatory regions of a diverse set of human UMOD orthologs to identify conserved binding motifs and corresponding position specific weight matrices. We further analyzed the predicted binding motifs by motif comparison, which identified transcription factors likely to bind these discovered motifs. Predicted transcription factors were then integrated with experimentally known protein-protein interactions available from public databases and tissue specific expression resources to delineate important regulators controlling UMOD expression. RESULTS: Analysis allowed the identification of a reliable set of binding motifs in the upstream regulatory regions of UMOD to build a high confidence compendium of transcription factors that could bind these motifs, such as GATA3, HNF1B, SP1, SMAD3, RUNX2 and KLF4. ENCODE deoxyribonuclease I hypersensitivity sites in the UMOD upstream region of the mouse kidney confirmed that some of these binding motifs were open to binding by predicted transcription factors. The transcription factor-transcription factor network revealed several highly connected transcription factors, such as SP1, SP3, TP53, POU2F1, RARB, RARA and RXRA, as well as the likely protein complexes formed between them. Expression levels of these transcription factors in the kidney suggest their central role in controlling UMOD expression. CONCLUSIONS: Our findings will form a map for understanding the regulation of uromodulin expression in health and disease.

Steketee, M. B., C. Oboudiyat, et al. "Regulation of intrinsic axon growth ability at retinal ganglion cell growth cones." Invest Ophthalmol Vis Sci. 2014 Jun 6;55(7):4369-77. doi: 10.1167/iovs.14-13882.

PURPOSE: Mammalian central nervous system neurons fail to regenerate after injury or disease, in part due to a progressive loss in intrinsic axon growth ability after birth. Whether lost axon growth ability is due to limited growth resources or to changes in the axonal growth cone is unknown. METHODS: Static and time-lapse images of purified retinal ganglion cells (RGCs) were analyzed for axon growth rate and growth cone morphology and dynamics without treatment and after manipulating Kruppel-like transcription factor (KLF) expression or applying mechanical tension. RESULTS: Retinal ganglion cells undergo a developmental switch in growth cone dynamics that mirrors the decline in postnatal axon growth rates, with increased filopodial adhesion and decreased lamellar protrusion area in postnatal axonal growth cones. Moreover, expressing growth-suppressive KLF4 or growth-enhancing KLF6 transcription factors elicits similar changes in postnatal growth cones that correlate with axon growth rates. Postnatal RGC axon growth rate is not limited by an inability to achieve axon growth rates similar to embryonic RGCs; indeed, postnatal axons support elongation rates up to 100-fold faster than postnatal axonal growth rates. Rather, the intrinsic capacity for rapid axon growth is due to both growth cone pausing and retraction, as well as to a slightly decreased ability to achieve rapid instantaneous rates of forward progression. Finally, we observed that RGC axon and dendrite growth are regulated independently in vitro. CONCLUSIONS: Together, these data support the hypothesis that intrinsic axon growth rate is regulated by an axon-specific growth program that differentially regulates growth cone motility.

Stuart, H. T., A. L. van Oosten, et al. "NANOG amplifies STAT3 activation and they synergistically induce the naive pluripotent program." Curr Biol. 2014 Feb 3;24(3):340-6. doi: 10.1016/j.cub.2013.12.040. Epub 2014 Jan 23.

Reprogramming of a differentiated cell back to a naive pluripotent identity is thought to occur by several independent mechanisms. Two such mechanisms include NANOG and activated STAT3 (pSTAT3), known master regulators of naive pluripotency acquisition [1-5]. Here, we investigated the relationship between NANOG and pSTAT3 during the establishment and maintenance of naive pluripotency. Surprisingly, we found that NANOG enhances LIF signal transduction, resulting in elevated pSTAT3. This is mediated, at least in part, by suppression of the expression of the LIF/STAT3 negative regulator SOCS3. We also discovered NANOG to be limiting for the expression of KLF4, a canonical "Yamanaka" reprogramming factor [6] and key pSTAT3 target [2, 7, 8]. KLF4 expression resulted from the codependent and synergistic action of NANOG and pSTAT3 in embryonic stem cells and during initiation of reprogramming. Additionally, within 48 hr, the combined actions of NANOG and pSTAT3 in a reprogramming context resulted in reactivation of genes associated with naive pluripotency. Importantly, we show that NANOG can be bypassed during reprogramming by exogenous provision of its downstream effectors, namely pSTAT3 elevation and KLF4 expression. In conclusion, we propose that mechanisms of reprogramming are linked, rather than independent, and are centered on a small number of genes, including NANOG.

Sureban, S. M., R. May, et al. "XMD8-92 inhibits pancreatic tumor xenograft growth via a DCLK1-dependent mechanism." Cancer Lett. 2014 Aug 28;351(1):151-61. doi: 10.1016/j.canlet.2014.05.011. Epub 2014 May 28.

XMD8-92 is a kinase inhibitor with anti-cancer activity against lung and cervical cancers, but its effect on pancreatic ductal

adenocarcinoma (PDAC) remains unknown. Doublecortin-like kinase1 (DCLK1) is upregulated in various cancers including PDAC. In this study, we showed that XMD8-92 inhibits AsPC-1 cancer cell proliferation and tumor xenograft growth. XMD8-92 treated tumors demonstrated significant downregulation of DCLK1 and several of its downstream targets (including c-MYC, KRAS, NOTCH1, ZEB1, ZEB2, SNAIL, SLUG, OCT4, SOX2, NANOG, KLF4, LIN28, VEGFR1, and VEGFR2) via upregulation of tumor suppressor miRNAs let-7a, miR-144, miR-200a-c, and miR-143/145; it did not however affect BMK1 downstream genes p21 and p53. These data taken together suggest that XMD8-92 treatment results in inhibition of DCLK1 and downstream oncogenic pathways (EMT, pluripotency, angiogenesis and anti-apoptotic), and is a promising chemotherapeutic agent against PDAC.

Sutiwisesak, R., N. Kitiyanant, et al. "Induced pluripotency enables differentiation of human nullipotent embryonal carcinoma cells N2102Ep." Biochim Biophys Acta. 2014 Nov;1843(11):2611-9. doi: 10.1016/j.bbamcr.2014.07.013. Epub 2014 Jul 30.

Embryonal carcinoma (EC) cells, which are considered to be malignant counterparts of embryonic stem cells, comprise the pluripotent stem cell component of teratocarcinomas, a form of testicular germ cell tumors (GCTs). Nevertheless, many established human EC cell lines are nullipotent with limited or no capacity to differentiate under normal circumstances. In this study, we tested whether an over-expression of Yamanaka's reprogramming factors OCT4, SOX2, c-MYC and KLF4 might enable differentiation of the human nullipotent EC cells N2102Ep. Using OCT4 knockdown differentiated N2102Ep cells, we are able to derive reprogrammed N2102Ep cell lines. The induced pluripotency of N2102Ep allows the cells to differentiate toward neural lineage by retinoic acid; the expression of SSEA3 and SSEA4 is down-regulated, whereas that of neural surface markers is up-regulated. Consistent with the up-regulation of neural surface markers, the expression of the master neuroectodermal transcription factor PAX6 is also induced in reprogrammed N2102Ep. We next investigated whether PAX6 might induce spontaneous differentiation of nullipotent stem cells N2102Ep. However, while an ectopic expression of PAX6 promotes differentiation of NTERA2, it induces cell death in N2102Ep. We nevertheless find that upon induction of retinoic acid, the reprogrammed N2102Ep cells form mature neuronal morphology similar to differentiated pluripotent stem cells NTERA2 as determined by TUJ1 expression, which is absent in N2102Ep parental cells. Altogether, we conclude that the nullipotent state of human EC cells can be reprogrammed to acquire a more relaxed state of differentiation potential by Yamanaka's factors.

Tahmasebi, S., M. Ghorbani, et al. "The SUMO conjugating enzyme Ubc9 is required for inducing and maintaining stem cell pluripotency." Stem Cells. 2014 Apr;32(4):1012-20. doi: 10.1002/stem.1600.

Sumoylation adds a small ubiquitin-like modifier (SUMO) polypeptide to the epsilon-amino group of a lysine residue. Reminiscent of ubiquitination, sumoylation is catalyzed by an enzymatic cascade composed of E1, E2, and E3. For sumoylation, this cascade uses Ubc9 (ubiquitin conjugating enzyme 9, now officially named ubiquitin conjugating enzyme E2I [UBE2I]) as the sole E2 enzyme. Here, we report that expression of endogenous Ubc9 increases during reprogramming of mouse embryonic fibroblasts (MEFs) into induced pluripotent stem (iPS) cells. In addition, this E2 enzyme is required for reprogramming as its suppression dramatically inhibits iPS cell induction. While Ubc9 knockdown does not affect survival of MEFs and immortalized fibroblasts, Ubc9 is essential for embryonic stem cell (ESC) survival. In addition, we have found that Ubc9 knockdown stimulates apoptosis in ESCs but not in MEFs. Furthermore, the knockdown decreases the expression of the well-known pluripotency marker Nanog and the classical reprogramming factors Klf4, Oct4, and Sox2 in ESCs. Together, these observations indicate that while dispensable for fibroblast survival, the sole SUMO E2 enzyme Ubc9 plays a critical role in reprogramming fibroblasts to iPS cells and maintaining ESC pluripotency.

Takenaka-Ninagawa, N., Y. Kawabata, et al. "Generation of rat-induced pluripotent stem cells from a new model of metabolic syndrome." PLoS One. 2014 Aug 11;9(8):e104462. doi: 10.1371/journal.pone.0104462. eCollection 2014.

We recently characterized DahlS.Z-Leprfa/Leprfa (DS/obese) rats, derived from a cross between Dahl salt-sensitive rats and Zucker rats, as a new animal model of metabolic syndrome (MetS). Although the phenotype of DS/obese rats is similar to that of humans with MetS, the pathophysiological and metabolic characteristics in each cell type remain to be clarified. Hence, the establishment of induced pluripotent stem cells (iPSCs) derived from MetS rats is essential for investigations of MetS in vitro. Reports of rat iPSCs (riPSCs), however, are few because of the difficulty of comparing to other rodents such as mouse. Recently, the advantage of using mesenchymal stromal cells (MSCs) as a cell source for generating iPSCs was described. We aimed to establish riPSCs from MSCs in adipose tissues of both DS/obese rats and their lean littermates, DahlS.Z-Lepr+/Lepr+ (DS/lean) rats using lentivirus vectors with only three factors Oct4, Klf4, and Sox2 without c-Myc. The morphology, gene expression profiles, and protein expression of established colonies showed embryonic stem cell (ESCs)-like properties, and the differentiation potential into cells from all three germ layers both in vitro and in vivo (teratomas). Both riPSCs became adipocytes after induction of adipogenesis by insulin, T3, and dexamethasone. Real-time PCR analysis also revealed that both riPSCs and the adipose tissue from DS/obese and DS/lean rats possess similar expression patterns of adipocyte differentiation-related genes. We succeeded in generating riPSCs effectively from MSCs of both DS/obese and DS/lean rats. These riPSCs may well serve as highly effective tools for the investigation of MetS pathophysiology in vitro.

Tam, W. L., D. F. O, et al. "Sox9 reprogrammed dermal fibroblasts undergo hypertrophic differentiation in vitro and trigger endochondral ossification in vivo." Cell Reprogram. 2014 Feb;16(1):29-39. doi: 10.1089/cell.2013.0060.

Strategies for bone regeneration are undergoing a paradigm shift, moving away from the replication of end-stage bone tissue and instead aiming to recapture the initial events of fracture repair. Although this is known to resemble endochondral bone formation, chondrogenic cell types with favorable proliferative and hypertrophic differentiation properties are lacking. Recent advances in cellular reprogramming have allowed the creation of alternative cell populations with specific properties through the forced expression of transcription factors. Herein, we investigated the in vitro hypertrophic differentiation and in vivo tissue formation capacity of induced chondrogenic cells (iChon cells) obtained through direct reprogramming. In vitro hypertrophic differentiation was detected in iChon cells that contained a doxycycline-inducible expression system for Klf4, cMyc, and Sox9. Furthermore, endochondral bone formation was detected after implantation in nude mice. The bone tissue was derived entirely from host origin, whereas cartilage tissue contained cells from both host and donor. The results obtained highlight the promise of cellular reprogramming for the creation of functional skeletal cells that can be used for novel bone healing strategies.

Terry, R. L. and S. D. Miller "Molecular control of monocyte development." Cell Immunol. 2014 Mar 12. pii: S0008-8749(14)00034-3. doi: 10.1016/j.cellimm.2014.02.008.

Monocyte development is a tightly regulated and multi-staged process, occurring through several defined progenitor cell intermediates. The key transcription factors, including PU.1, IRF8 and KLF4, growth factors, such as M-CSF and IL-34 and cytokines that drive monocyte development from hematopoietic progenitor cells are well defined. However, the molecular controls that direct differentiation into the Ly6Chi inflammatory and Ly6Clo monocyte

subsets are yet to be completely elucidated. This review will provide a summary of the transcriptional regulation of monocyte development. We will also discuss how these molecular controls are also critical for microglial development despite their distinct haematopoetic origins. Furthermore, we will examine recent breakthroughs in defining mechanisms that promote differentiation of specific monocyte subpopulations.

Tiemann, U., A. G. Marthaler, et al. "Counteracting Activities of OCT4 and KLF4 during Reprogramming to Pluripotency." Stem Cell Reports. 2014 Feb 20;2(3):351-65. doi: 10.1016/j.stemcr.2014.01.005. eCollection 2014 Mar 11.

Differentiated cells can be reprogrammed into induced pluripotent stem cells (iPSCs) after overexpressing four transcription factors, of which Oct4 is essential. To elucidate the role of Oct4 during reprogramming, we investigated the immediate transcriptional response to inducible Oct4 overexpression in various somatic murine cell types using microarray analysis. By downregulating somatic-specific genes, Oct4 induction influenced each transcriptional program in a unique manner. A significant upregulation of pluripotent markers could not be detected. Therefore, OCT4 facilitates reprogramming by interfering with the somatic transcriptional network rather than by directly initiating a pluripotent gene-expression program. Finally, Oct4 overexpression upregulated the gene Mgarp in all the analyzed cell types. Strikingly, Mgarp expression decreases during the first steps of reprogramming due to a KLF4-dependent inhibition. At later stages, OCT4 counteracts the repressive activity of KLF4, thereby enhancing Mgarp expression. We show that this temporal expression pattern is crucial for the efficient generation of iPSCs.

Tomas, J., J. Reygner, et al. "Early colonizing Escherichia coli elicits remodeling of rat colonic epithelium shifting toward a new homeostatic state." ISME J. 2014 Jul 11. doi: 10.1038/ismej.2014.111.

We investigated the effects of early colonizing bacteria on the colonic epithelium. We isolated dominant bacteria, Escherichia coli, Enterococcus faecalis, Lactobacillus intestinalis, Clostridium innocuum and a novel Fusobacterium spp., from the intestinal contents of conventional suckling rats and transferred them in different combinations into germfree (GF) adult rats. Animals were investigated after various times up to 21 days. Proliferative cell markers (Ki67, proliferating cell nuclear antigen, phospho-histone H3, cyclin A) were higher in rats monocolonized with E. coli than in GF at all time points, but not in rats monocolonized with E. faecalis. The mucin content of goblet cells declined shortly after E. coli administration whereas the mucus layer doubled in thickness. Fluorescence in situ hybridization analyses revealed that E. coli resides in this mucus layer. The epithelial mucin content progressively returned to baseline, following an increase in KLF4 and in the cell cycle arrest-related proteins p21CIP1 and p27KIP1. Markers of colonic differentiated cells involved in electrolyte (carbonic anhydrase II and slc26A3) and water (aquaglyceroporin3 (aqp3)) transport, and secretory responses to carbachol were modulated after E. coli inoculation suggesting that ion transport dynamics were also affected. The colonic responses to simplified microbiotas differed substantially according to whether or not E. coli was combined with the other four bacteria. Thus, proliferation markers increased substantially when E. coli was in the mix, but very much less when it was absent. This work demonstrates that a pioneer strain of E. coli elicits sequential epithelial remodeling affecting the structure, mucus layer and ionic movements and suggests this can result in a microbiota-compliant state.The ISME Journal advance online publication, 11 July 2014; doi:10.1038/ismej.2014.111.

Toth, A., K. Fodor, et al. "Generation of induced pluripotent stem cells by using a mammalian artificial chromosome expression system." Acta Biol Hung. 2014 Sep;65(3):331-45. doi: 10.1556/ABiol.65.2014.3.9.

Direct reprogramming of mouse fibroblasts into induced pluripotent stem cells (iPS) was achieved recently by overexpression of four transcription factors encoded by retroviral vectors. Most of the virus vectors, however, may cause insertional mutagenesis in the host genome and may also induce tumor formation. Therefore, it is very important to discover novel and safer, non-viral reprogramming methods. Here we describe the reprogramming of somatic cells into iPS cells by a novel protein-based technique. Engineered Oct4, Sox2 and Klf4 transcription factors carrying an N-terminal Flag-tag and a C-terminal polyarginine tail were synthesized by a recently described mammalian artificial chromosome expression system (ACEs). This system is suitable for the high-level production of recombinant proteins in mammalian tissue culture cells. Recombinant proteins produced in this system contain all the post-translational modifications essential for the stability and the authentic function of the proteins. The engineered Oct4, Sox2 and Klf4 proteins efficiently induced the reprogramming of mouse embryonic fibroblasts by means of protein transduction. This novel method allows for the generation of iPS cells, which may be suitable for therapeutic applications in the future.

Tsukiyama, T. and Y. Ohinata "A modified EpiSC culture condition containing a GSK3 inhibitor can support germline-competent pluripotency in mice." PLoS One. 2014 Apr 15;9(4):e95329. doi: 10.1371/journal.pone.0095329. eCollection 2014.

Embryonic stem cells (ESCs) can contribute to the tissues of chimeric animals, including the germline. By contrast, epiblast stem cells (EpiSCs) barely contribute to chimeras. These two types of cells are established and maintained under different culture conditions. Here, we show that a modified EpiSC culture condition containing the GSK3 inhibitor CHIR99021 can support a germline-competent pluripotent state that is intermediate between ESCs and EpiSCs. When ESCs were cultured under a modified condition containing bFGF, Activin A, and CHIR99021, they converted to intermediate pluripotent stem cells (INTPSCs). These INTPSCs were able to form teratomas in vivo and contribute to chimeras by blastocyst injection. We also induced formation of INTPSCs (iINTPSCs) from mouse embryonic fibroblasts by exogenous expression of four reprogramming factors, Oct3/4, Sox2, Klf4, and c-Myc, under the INTPSC culture condition. These iINTPSCs contributed efficiently to chimeras, including the germline, by blastocyst injection. The INTPSCs exhibited several characteristic properties of both ESCs and EpiSCs. Our results suggest that the modified EpiSC culture condition can support growth of cells that meet the most stringent criteria for pluripotency, and that germline-competent pluripotency may depend on the activation state of Wnt signaling.

Tugal, D., M. K. Jain, et al. Endothelial KLF4: crippling vascular injury?, J Am Heart Assoc. 2014 Feb 26;3(1):e000769. doi: 10.1161/JAHA.113.000769.

Udagawa, K. and T. Ohyama "Positions of pluripotency genes and hepatocyte-specific genes in the nucleus before and after mouse ES cell differentiation." Genet Mol Res. 2014 Mar 24;13(1):1979-88. doi: 10.4238/2014.March.24.2.

Spatial positioning of genes in the cell nucleus plays an important role in the regulation of genomic functions. Evidence for changes in gene positioning associated with transcriptional activity has been reported. However, our understanding of this phenomenon is still quite limited. We examined how pluripotency genes and hepatocyte-specific genes behave during the differentiation of mouse embryonic stem (ES) cells into hepatocytes, by targeting the loci of the Klf4, Nanog, Oct4, Sox2, Cyp7alpha1, Pck1, Tat, and Tdo2 genes, and using three-dimensional fluorescence in situ hybridization analyses. We found that each gene has a distinctly inherent localization profile in the ES cell nucleus. During differentiation, the Klf4, Nanog, Oct4, Cyp7alpha1, Pck1, and Tat loci shifted toward the nuclear center, while the Sox2 and Tdo2 loci

shifted toward the periphery. The Klf4, Nanog, Oct4, and Tdo2 seem to prefer the outer regions, rather than the inner regions, when they are active. We also found that the radial positioning of the focused genes in the hepatocyte cell nucleus was highly correlated with the local GC content and the gene density of the surrounding region, but not with gene activity.

Varshney, A., S. K. Ramakrishnan, et al. "Global expression profile of telomerase-associated genes in HeLa cells." Gene. 2014 Sep 1;547(2):211-7. doi: 10.1016/j.gene.2014.06.018. Epub 2014 Jun 11.

Telomerase is a specialized nucleoprotein enzyme complex that maintains the telomere length. The telomerase reverse transcriptase (TERT) is the catalytically active component of the telomerase complex. In humans, the protein component (hTERT) and RNA component (hTR) are found to differentially express in cancer cells. In contrast to differentiated cells, most of the cancer cells overexpress hTERT, which is needed to maintain the proliferative potential of cells. The overexpression of telomerase is not proportionate to telomere length in cancer cells, suggesting that the immortalizing phenotype can be mediated through other factors in addition to telomere length. To investigate the role of hTERT in immortalizing process, loss of gene function studies were carried out. Short interfering RNA (siRNA) and short hairpin RNA (shRNA) against hTERT showed the reduction of hTERT transcript, reduction of telomerase activity and alteration of gene expression in HeLa cells. The molecular basis of proliferative capacity of hTERT was investigated by gene expression microarray. Analysis of microarray data for HeLa cells following siRNA and shRNA mediated knockdown of hTERT showed that 80 genes were upregulated and 73 genes downregulated. Out of these, 37 genes are known to be involved in cancer. Further analyses of previously known genes involved in cancer like KLF4, FGF2, IRF-9 and PLAU by Real Time PCR showed their upregulation. We are documenting for the first time the effect of knocking down hTERT on expression of KLF4 and FGF2. Interestingly, it has been earlier reported that KLF4 and FGF2 up-regulate the expression of hTERT in cancer cells. This suggests that hTERT may be subject to its own auto-regulatory effects.

Villarreal, G., Jr., A. Chatterjee, et al. "Pharmacological regulation of SPARC by lovastatin in human trabecular meshwork cells." Invest Ophthalmol Vis Sci. 2014 Mar 19;55(3):1657-65. doi: 10.1167/iovs.13-12712.

PURPOSE: Statins have been shown to increase aqueous outflow facility. The matricellular protein SPARC (secreted protein acidic and rich in cysteine) is a critical mediator of aqueous outflow and intraocular pressure (IOP). Here, we examine the effects of lovastatin on SPARC expression in trabecular meshwork (TM) cells, exploring the molecular mechanisms involved. METHODS: Primary cultured human TM cells were incubated for 24, 48, and 72 hours with 10 muM lovastatin. In separate cultures, media was supplemented with either farnesyl pyrophosphate (FPP) or geranylgeranyl pyrophosphate (GGPP) for the duration of the 72-hour time point experiment. Trabecular meshwork cells were also pretreated for 24 hours with lovastatin followed by 24-hour stimulation with 3 ng/mL TGF-beta2. Cell lysates and media were harvested and relative mRNA and protein level changes were determined. Kruppel-like factor 4 (KLF4) localization in normal human anterior segments was examined by immunofluorescence. Adenovirus expressing human KLF4 was used and relative changes in SPARC mRNA and protein levels were assessed. RESULTS: Incubating TM cells with lovastatin suppressed SPARC mRNA and protein levels. This effect was reversed upon media supplementation with GGPP but not FPP. Pretreating cells with lovastatin inhibited TGF-beta2 induction of SPARC. The KLF4 transcription factor was expressed throughout the TM and the inner and outer walls of Schlemm's canal. Lovastatin treatment upregulated KLF4 mRNA and protein levels. Overexpression of KLF4 downregulated SPARC expression. CONCLUSIONS: Collectively, our data identify lovastatin as an important pharmacological suppressor of SPARC expression in TM cells, and provide further insight into the molecular mechanisms mediating statin enhancement of aqueous outflow facility.

Wallner, S., M. Grandl, et al. "Monocyte to macrophage differentiation goes along with modulation of the plasmalogen pattern through transcriptional regulation." PLoS One. 2014 Apr 8;9(4):e94102. doi: 10.1371/journal.pone.0094102. eCollection 2014.

BACKGROUND: Dysregulation of monocyte-macrophage differentiation is a hallmark of vascular and metabolic diseases and associated with persistent low grade inflammation. Plasmalogens represent ether lipids that play a role in diabesity and previous data show diminished plasmalogen levels in obese subjects. We therefore analyzed transcriptomic and lipidomic changes during monocyte-macrophage differentiation in vitro using a bioinformatic approach. METHODS: Elutriated monocytes from 13 healthy donors were differentiated in vitro to macrophages using rhM-CSF under serum-free conditions. Samples were taken on days 0, 1, 4 and 5 and analyzed for their lipidomic and transcriptomic profiles. RESULTS: Gene expression analysis showed strong regulation of lipidome-related transcripts. Enzymes involved in fatty acid desaturation and elongation were increasingly expressed, peroxisomal and ER stress related genes were induced. Total plasmalogen levels remained unchanged, while the PE plasmalogen species pattern became more similar to circulating granulocytes, showing decreases in PUFA and increases in MUFA. A partial least squares discriminant analysis (PLS/DA) revealed that PE plasmalogens discriminate the stage of monocyte-derived macrophage differentiation. Partial correlation analysis could predict novel potential key nodes including DOCK1, PDK4, GNPTAB and FAM126A that might be involved in regulating lipid and especially plasmalogen homeostasis during differentiation. An in silico transcription analysis of lipid related regulation revealed known motifs such as PPAR-gamma and KLF4 as well as novel candidates such as NFY, RNF96 and Zinc-finger proteins. CONCLUSION: Monocyte to macrophage differentiation goes along with profound changes in the lipid-related transcriptome. This leads to an induction of fatty-acid desaturation and elongation. In their PE-plasmalogen profile macrophages become more similar to granulocytes than monocytes, indicating terminal phagocytic differentiation. Therefore PE plasmalogens may represent potential biomarkers for cell activation. For the underlying transcriptional network we were able to predict a range of novel central key nodes and underlying transcription factors using a bioinformatic approach.

Wasik, A. M., J. Grabarek, et al. "Reprogramming and carcinogenesis--parallels and distinctions." Int Rev Cell Mol Biol. 2014;308:167-203. doi: 10.1016/B978-0-12-800097-7.00005-1.

Rapid progress made in various areas of regenerative medicine in recent years occurred both at the cellular level, with the Nobel prize-winning discovery of reprogramming (generation of induced pluripotent stem (iPS) cells) and also at the biomaterial level. The use of four transcription factors, Oct3/4, Sox2, c-Myc, and Klf4 (called commonly "Yamanaka factors") for the conversion of differentiated cells, back to the pluripotent/embryonic stage, has opened virtually endless and ethically acceptable source of stem cells for medical use. Various types of stem cells are becoming increasingly popular as starting components for the development of replacement tissues, or artificial organs. Interestingly, many of the transcription factors, key to the maintenance of stemness phenotype in various cells, are also overexpressed in cancer (stem) cells, and some of them may find the use as prognostic factors. In this review, we describe various methods of iPS creation, followed by overview of factors known to interfere with the efficiency of reprogramming. Next, we discuss similarities between cancer stem cells and various stem cell types. Final paragraphs are dedicated to interaction of biomaterials with tissues, various adverse reactions generated as a

result of such interactions, and measures available, that allow for mitigation of such negative effects.

Watson, J. E., N. A. Patel, et al. "Comparison of Markers and Functional Attributes of Human Adipose-Derived Stem Cells and Dedifferentiated Adipocyte Cells from Subcutaneous Fat of an Obese Diabetic Donor." Adv Wound Care (New Rochelle). 2014 Mar 1;3(3):219-228.

Objective: Adipose tissue is a robust source of adipose-derived stem cells (ADSCs) that may be able to provide secreted factors that promote the ability of wounded tissue to heal. However, adipocytes also have the potential to dedifferentiate in culture to cells with stem cell-like properties that may improve their behavior and functionality for certain applications. Approach: ADSCs are adult mesenchymal stem cells that are cultured from the stromal vascular fraction of adipose tissue. However, adipocytes are capable of dedifferentiating into cells with stem cell properties. In this case study, we compare ADSC and dedifferentiated fat (DFAT) cells from the same patient and fat depot for mesenchymal cell markers, embryonic stem cell markers, ability to differentiate to adipocytes and osteoblasts, senescence and telomerase levels, and ability of conditioned media (CM) to stimulate migration of human dermal fibroblasts (HDFs). Innovation and Conclusions: ADSCs and DFAT cells displayed identical levels of CD90, CD44, CD105, and were CD34- and CD45-negative.

West, J. A., A. Cook, et al. "Nucleosomal occupancy changes locally over key regulatory regions during cell differentiation and reprogramming." Nat Commun. 2014 Aug 27;5:4719. doi: 10.1038/ncomms5719.

Chromatin structure determines DNA accessibility. We compare nucleosome occupancy in mouse and human embryonic stem cells (ESCs), induced-pluripotent stem cells (iPSCs) and differentiated cell types using MNase-seq. To address variability inherent in this technique, we developed a bioinformatic approach to identify regions of difference (RoD) in nucleosome occupancy between pluripotent and somatic cells. Surprisingly, most chromatin remains unchanged; a majority of rearrangements appear to affect a single nucleosome. RoDs are enriched at genes and regulatory elements, including enhancers associated with pluripotency and differentiation. RoDs co-localize with binding sites of key developmental regulators, including the reprogramming factors Klf4, Oct4/Sox2 and c-Myc. Nucleosomal landscapes in ESC enhancers are extensively altered, exhibiting lower nucleosome occupancy in pluripotent cells than in somatic cells. Most changes are reset during reprogramming. We conclude that changes in nucleosome occupancy are a hallmark of cell differentiation and reprogramming and likely identify regulatory regions essential for these processes.

Yamasaki, S., Y. Taguchi, et al. "Generation of human induced pluripotent stem (Ips) cells in serum- and feeder-free defined culture and TGF-Beta1 regulation of pluripotency." PLoS One. 2014 Jan 29;9(1):e87151. doi: 10.1371/journal.pone.0087151. eCollection 2014.

Human Embryonic Stem cells (hESCs) and human induced Pluripotent Stem cells (hiPSCs) are commonly maintained on inactivated mouse embryonic fibroblast as feeder cells in medium supplemented with FBS or proprietary replacements. Use of culture medium containing undefined or unknown components has limited the development of applications for pluripotent cells because of the relative lack of knowledge regarding cell responses to differentiating growth factors. In addition, there is no consensus as to the optimal formulation, or the nature of the cytokine requirements of the cells to promote their self-renewal and inhibit their differentiation. In this study, we successfully generated hiPSCs from human dental pulp cells (DPCs) using Yamanaka's factors (Oct3/4, Sox2, Klf4, and c-Myc) with retroviral vectors in serum- and feeder-free defined culture conditions. These hiPSCs retained the property of self-renewal as evaluated by the expression of self-renewal marker genes and proteins, morphology, cell growth rates, and pluripotency evaluated by differentiation into derivatives of all three primary germ layers in vitro and in vivo. In this study, we found that TGF-beta1 increased the expression levels of pluripotency markers in a dose-dependent manner. However, increasing doses of TGF-beta1 suppressed the growth rate of hiPSCs cultured under the defined conditions. Furthermore, over short time periods the hiPSCs cultured in hESF9 or hESF9T exhibited similar morphology, but hiPSCs maintained in hESF9 could not survive beyond 30 passages. This result clearly confirmed that hiPSCs cultured in hESF9 medium absolutely required TGF-beta1 to maintain pluripotency. This simple serum-free adherent monoculture system will allow us to elucidate the cell responses to growth factors under defined conditions and can eliminate the risk might be brought by undefined pathogens.

Yoshida, T., M. Yamashita, et al. "Kruppel-like Factor 4 Regulates Isoproterenol-induced Cardiac Hypertrophy by Modulating Myocardin Expression and Activity." J Biol Chem. 2014 Aug 6. pii: jbc.M114.582809.

Kruppel-like factor 4 (KLF4) plays an important role in vascular diseases, including atherosclerosis and vascular injury. Although KLF4 is expressed in the heart in addition to vascular cells, the role of KLF4 in cardiac disease has not been fully determined yet. The goals of the present study were to investigate the role of KLF4 in cardiac hypertrophy and to determine the underlying mechanisms. Cardiomyocyte-specific Klf4 knockout (CM Klf4 KO) mice were generated by the Cre/LoxP technique. Cardiac hypertrophy was induced by chronic infusion of beta-adrenoceptor agonist, isoproterenol (ISO). Results showed that ISO-induced cardiac hypertrophy was enhanced in CM Klf4 KO mice, as compared to control mice. Accelerated cardiac hypertrophy in CM Klf4 KO mice was accompanied by the augmented cellular enlargement of cardiomyocytes, as well as the exaggerated expression of fetal cardiac genes including atrial natriuretic factor (Nppa). Additionally, induction of myocardin, a transcriptional co-factor regulating fetal cardiac genes, was enhanced in CM Klf4 KO mice. Interestingly, KLF4 regulated Nppa expression by modulating the expression and activity of myocardin, providing a mechanical basis for accelerated cardiac hypertrophy in CM Klf4 KO mice. Moreover, we showed that KLF4 mediated anti-hypertrophic effect of trichostatin A, a histone deacetylase inhibitor, because ISO-induced cardiac hypertrophy in CM Klf4 KO mice was attenuated by olmesartan, an angiotensin II type 1 antagonist, but not by trichostatin A. These results provide novel evidence that KLF4 is a regulator of cardiac hypertrophy by modulating the expression and the activity of myocardin.

Zohre, S., N. K. Kazem, et al. "Trichostatin A-induced Apoptosis is Mediated by Kruppel-like Factor 4 in Ovarian and Lung Cancer." Asian Pac J Cancer Prev. 2014;15(16):6581-6.

BACKGROUND: The istone deacetylase (HDAC) inhibitor trichostatin A (TSA) is known to mediate the regulation of gene expression and antiproliferation activity in cancer cells. Kruppel-like factor 4 (klf4) is a zinc finger- containing transcription factor of the SP/KLF family, that is expressed in a variety of tissues and regulates cell proliferation, differentiation, tumorigenesis, and apoptosis. It may either either function as a tumor suppressor or an oncogene depending on genetic context of tumors. AIMS: In this study, we tested the possibility that TSA may increase klf4 expression and cancer cell growth inhibition and apoptosis in SKOV-3 and A549 cells. The cytotoxicity of TSA was determined using the MTT assay test, while klf4 gene expression was assessed by real time PCR andto ability of TSA to induce apoptosis using a Vybrant Apoptosis Assay kit. RESULTS: Our results showed that TSA exerted dose and time dependent cytotoxicity effect on SKOV-3 and A549 cells. Moreover TSA up-regulated klf4 expression. Flow cytometric analysis demonstrated that apoptosis was increased after TSA treatment.

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