

Embryonic Stem Cell Research Literatures

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Abstract: The stem cell is the origin of an organism's life that has the potential to develop into many different types of cells in life bodies. In many tissues stem cells serve as a sort of internal repair system, dividing essentially without limit to replenish other cells as long as the person or animal is still alive. When a stem cell divides, each new cell has the potential either to remain a stem cell or become another type of cell with a more specialized function, such as a red blood cell or a brain cell. This article introduces recent research reports as references in the related studies.

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Key words: stem cell; embryonic; life; research; literature

Introduction

The stem cell is the origin of an organism's life that has the potential to develop into many different types of cells in life bodies. In many tissues stem cells serve as a sort of internal repair system, dividing essentially without limit to replenish other cells as long as the person or animal is still alive. When a stem cell divides, each new cell has the potential either to remain a stem cell or become another type of cell with a more specialized function, such as a red blood cell or a brain cell.

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

Alajem, A., A. Biran, et al. "Differential association of chromatin proteins identifies BAF60a/SMARCD1 as a regulator of embryonic stem cell differentiation." *Cell Rep.* 2015 Mar 31;10(12):2019-31. doi: [10.1016/j.celrep.2015.02.064](https://doi.org/10.1016/j.celrep.2015.02.064). Epub 2015 Mar 26.

Embryonic stem cells (ESCs) possess a distinct chromatin conformation maintained by specialized chromatin proteins. To identify chromatin regulators in ESCs, we developed a simple biochemical assay named D-CAP (differential chromatin-associated proteins), using brief micrococcal nuclease digestion of chromatin, followed by liquid chromatography tandem mass spectrometry (LC-MS/MS). Using D-CAP, we identified several differentially chromatin-associated proteins between undifferentiated and differentiated ESCs, including the chromatin remodeling protein SMARCD1. SMARCD1 depletion in ESCs led to altered chromatin and enhanced endodermal differentiation. Gene expression and chromatin immunoprecipitation sequencing (ChIP-seq) analyses suggested that SMARCD1 is both an activator and a repressor and is enriched at developmental regulators and that its chromatin binding coincides with H3K27me3.

SMARCD1 knockdown caused H3K27me3 redistribution and increased H3K4me3 around the transcription start site (TSS). One of the identified SMARCD1 targets was Klf4. In SMARCD1-knockdown clones, KLF4, as well as H3K4me3 at the Klf4 locus, remained high and H3K27me3 was abolished. These results propose a role for SMARCD1 in restricting pluripotency and activating lineage pathways by regulating H3K27 methylation.

Amer, L. D., A. Holtzinger, et al. "Enzymatically degradable poly(ethylene glycol) hydrogels for the 3D culture and release of human embryonic stem cell derived pancreatic precursor cell aggregates." *Acta Biomater.* 2015 Aug;22:103-10. doi: [10.1016/j.actbio.2015.04.013](https://doi.org/10.1016/j.actbio.2015.04.013). Epub 2015 Apr 22.

This study aimed to develop a three dimensional culture platform for aggregates of human embryonic stem cell (hESC)-derived pancreatic progenitors that enables long-term culture, maintains aggregate size and morphology, does not adversely affect differentiation and provides a means for aggregate recovery. A platform was developed with poly(ethylene glycol) hydrogels containing collagen type I, for cell-matrix interactions, and peptide crosslinkers, for facile recovery of aggregates. The platform was first demonstrated with RIN-m5F cells, showing encapsulation and subsequent release of single cells and aggregates without adversely affecting viability. Aggregates of hESC-derived pancreatic progenitors with an effective diameter of 82 (15)µm were either encapsulated in hydrogels or cultured in suspension for 28 days. At day 14, aggregate viability was maintained in the hydrogels, but significantly reduced (88%) in suspension culture. However by day 28, viability was reduced under both culture conditions. Aggregate size was maintained in the hydrogels, but in suspension was significantly higher (

approximately 2-fold) by day 28. The ability to release aggregates followed by a second enzyme treatment to achieve single cells enabled assessment by flow cytometry. Prior to encapsulation, there were 39% Pdx1(+)/Nkx6.1(+) cells, key endocrine markers required for beta-cell maturation. The fraction of doubly positive cells was not affected in hydrogels but was slightly and significantly lower in suspension culture by 28 days. In conclusion, we demonstrate that a MMP-sensitive PEG hydrogel containing collagen type I is a promising platform for hESC-derived pancreatic progenitors that maintains viable aggregates, aggregate size, and progenitor state and offers facile recovery of aggregates.

Bertero, A., P. Madrigal, et al. "Activin/nodal signaling and NANOG orchestrate human embryonic stem cell fate decisions by controlling the H3K4me3 chromatin mark." *Genes Dev.* 2015 Apr 1;29(7):702-17. doi: 10.1101/gad.255984.114. Epub 2015 Mar 24.

Stem cells can self-renew and differentiate into multiple cell types. These characteristics are maintained by the combination of specific signaling pathways and transcription factors that cooperate to establish a unique epigenetic state. Despite the broad interest of these mechanisms, the precise molecular controls by which extracellular signals organize epigenetic marks to confer multipotency remain to be uncovered. Here, we use human embryonic stem cells (hESCs) to show that the Activin-SMAD2/3 signaling pathway cooperates with the core pluripotency factor NANOG to recruit the DPY30-COMPASS histone modifiers onto key developmental genes. Functional studies demonstrate the importance of these interactions for correct histone 3 Lys4 trimethylation and also self-renewal and differentiation. Finally, genetic studies in mice show that Dpy30 is also necessary to maintain pluripotency in the pregastrulation embryo, thereby confirming the existence of similar regulations in vivo during early embryonic development. Our results reveal the mechanisms by which extracellular factors coordinate chromatin status and cell fate decisions in hESCs.

Bruin, J. E., N. Saber, et al. "Treating diet-induced diabetes and obesity with human embryonic stem cell-derived pancreatic progenitor cells and antidiabetic drugs." *Stem Cell Reports.* 2015 Apr 14;4(4):605-20. doi: 10.1016/j.stemcr.2015.02.011. Epub 2015 Mar 19.

Human embryonic stem cell (hESC)-derived pancreatic progenitor cells effectively reverse hyperglycemia in rodent models of type 1 diabetes, but their capacity to treat type 2 diabetes has not been reported. An immunodeficient model of type 2 diabetes was generated by high-fat diet (HFD) feeding

in SCID-beige mice. Exposure to HFDs did not impact the maturation of macroencapsulated pancreatic progenitor cells into glucose-responsive insulin-secreting cells following transplantation, and the cell therapy improved glucose tolerance in HFD-fed transplant recipients after 24 weeks. However, since diet-induced hyperglycemia and obesity were not fully ameliorated by transplantation alone, a second cohort of HFD-fed mice was treated with pancreatic progenitor cells combined with one of three antidiabetic drugs. All combination therapies rapidly improved body weight and co-treatment with either sitagliptin or metformin improved hyperglycemia after only 12 weeks. Therefore, a stem cell-based therapy may be effective for treating type 2 diabetes, particularly in combination with antidiabetic drugs.

Corradi, S., E. Dakou, et al. "Morphological observation of embryoid bodies completes the in vitro evaluation of nanomaterial embryotoxicity in the embryonic stem cell test (EST)." *Toxicol In Vitro.* 2015 Jun 17. pii: S0887-2333(15)00151-4. doi: 10.1016/j.tiv.2015.06.015.

The wide and frequent use of engineered nanomaterials (NMs) raises serious concerns about their safety for human health. Our aim is to evaluate the embryotoxic potential of silver, uncoated and coated zinc oxide, titanium dioxide and silica NMs through the embryonic stem cell test (EST). EST is a validated in vitro assay that permits classification of chemicals into three classes (non, weakly or strongly embryotoxic). Because of the peculiar physico-chemical characteristics of NMs, we first adapted and simplified the differentiation protocol. To verify the efficiency of this adapted protocol we screened 3 well-characterized chemicals (5-fluorouracil, hydroxyurea and saccharin). Next, we assessed the embryotoxic potential of NMs. Our data showed that silver NM is classified as a strong embryotoxic compound, while coated and uncoated zinc oxide, titanium and silica NMs as weak embryotoxic compounds. In addition, we observed daily the formation and growth of embryoid bodies (EBs). We showed that multiple EBs formed in each well starting from 50µg/ml of SiO₂ while EB formation was inhibited starting from 20µg/ml of ZnO NMs. This has never been reported with chemicals and could pose a risk of wrongly evaluating the NMs embryotoxic potential. For NMs, morphological observation of EBs can provide valuable information on early differentiation effects. Finally, we suggest that the prediction model should be revised for the assessment of NMs embryotoxicity.

Desai, N., P. Rambhia, et al. "Human embryonic stem cell cultivation: historical perspective and evolution of

xeno-free culture systems." Reprod Biol Endocrinol. 2015 Feb 22;13:9. doi: 10.1186/s12958-015-0005-4.

Human embryonic stem cells (hESC) have emerged as attractive candidates for cell-based therapies that are capable of restoring lost cell and tissue function. These unique cells are able to self-renew indefinitely and have the capacity to differentiate in to all three germ layers (ectoderm, endoderm and mesoderm). Harnessing the power of these pluripotent stem cells could potentially offer new therapeutic treatment options for a variety of medical conditions. Since the initial derivation of hESC lines in 1998, tremendous headway has been made in better understanding stem cell biology and culture requirements for maintenance of pluripotency. The approval of the first clinical trials of hESC cells for treatment of spinal cord injury and macular degeneration in 2010 marked the beginning of a new era in regenerative medicine. Yet it was clearly recognized that the clinical utility of hESC transplantation was still limited by several challenges. One of the most immediate issues has been the exposure of stem cells to animal pathogens, during hESC derivation and during in vitro propagation. Initial culture protocols used co-culture with inactivated mouse fibroblast feeder (MEF) or human feeder layers with fetal bovine serum or alternatively serum replacement proteins to support stem cell proliferation. Most hESC lines currently in use have been exposed to animal products, thus carrying the risk of xeno-transmitted infections and immune reaction. This mini review provides a historic perspective on human embryonic stem cell culture and the evolution of new culture models. We highlight the challenges and advances being made towards the development of xeno-free culture systems suitable for therapeutic applications.

Ehnes, D. D., R. M. Geransar, et al. "Exogenous nitric oxide enhances calcification in embryonic stem cell-derived osteogenic cultures." Differentiation. 2015 Mar-Apr;89(3-4):97-103. doi: 10.1016/j.diff.2015.02.001. Epub 2015 Apr 27.

While the involvement of nitric oxide in bone formation, homeostasis and healing has been extensively characterized, its role in directing pluripotent stem cells to the osteogenic lineage has not been described. Yet, the identification of chemical inducers that improve differentiation output to a particular lineage is highly valuable to the development of such cells for the cell-based treatment of osteo-degenerative diseases. This study aimed at investigating the instructive role of nitric oxide (NO) and its synthesizing enzymes on embryonic stem cell (ESC) osteogenic differentiation. Our findings showed that NO levels may support osteogenesis, but that the

effect of nitric oxide on osteoblast differentiation may be specific to a particular time phase during the development of osteoblasts in vitro. Endogenously, nitric oxide was specifically secreted by osteogenic cultures during the calcification period. Simultaneously, messenger RNAs for both the endothelial and inducible nitric oxide synthase isoforms (eNOS and iNOS) were upregulated during this late phase development. However, the specific eNOS inhibitor L-N(5)-(1-Iminoethyl)ornithine dihydrochloride attenuated calcification more so than the specific iNOS inhibitor diphenyleneiodonium. Exogenous stage-specific supplementation of culture medium with the NO donor S-nitroso-N-acetylpenicillamine increased the percentage of cells differentiating into osteoblasts and enhanced calcification. Our results point to a primary role for eNOS as a pro-osteogenic trigger in ESC differentiation and expand on the variety of supplements that may be used to direct ESC fate to the osteogenic lineage, which will be important in the development of cell-based therapies for osteo-degenerative diseases.

Elert-Dobkowska, E., J. C. Hennings, et al. "Multiplex ligation-dependent probe amplification for identification of correctly targeted murine embryonic stem cell clones." Anal Biochem. 2015 Apr 1;474:35-7. doi: 10.1016/j.ab.2015.01.007. Epub 2015 Jan 20.

Following locus-specific genome editing of mouse embryonic stem cells (ESCs), the identification of correctly targeted clones remains a challenge. We applied multiplex ligation-dependent probe amplification (MLPA) to screen for homologous recombination-based genomic integration of a knockout construct in which part of a gene is deleted. All candidate ESCs thereby identified were subsequently validated by conventional methods. Thus, MLPA represents a highly reliable as well as cost- and time-efficient alternative to currently applied methods such as Southern blotting and polymerase chain reaction (PCR)-based approaches. It is also applicable to knockin recombination strategies and compatible with the CRISPR/Cas9 system and other genome editing strategies.

Etchegaray, J. P., L. Chavez, et al. "The histone deacetylase SIRT6 controls embryonic stem cell fate via TET-mediated production of 5-hydroxymethylcytosine." Nat Cell Biol. 2015 May;17(5):545-57. doi: 10.1038/ncb3147. Epub 2015 Apr 27.

How embryonic stem cells (ESCs) commit to specific cell lineages and yield all cell types of a fully formed organism remains a major question. ESC differentiation is accompanied by large-scale histone

and DNA modifications, but the relations between these epigenetic categories are not understood. Here we demonstrate the interplay between the histone deacetylase sirtuin 6 (SIRT6) and the ten-eleven translocation enzymes (TETs). SIRT6 targets acetylated histone H3 at Lys 9 and 56 (H3K9ac and H3K56ac), while TETs convert 5-methylcytosine into 5-hydroxymethylcytosine (5hmC). ESCs derived from Sirt6 knockout (S6KO) mice are skewed towards neuroectoderm development. This phenotype involves derepression of OCT4, SOX2 and NANOG, which causes an upregulation of TET-dependent production of 5hmC. Genome-wide analysis revealed neural genes marked with 5hmC in S6KO ESCs, thereby implicating TET enzymes in the neuroectoderm-skewed differentiation phenotype. We demonstrate that SIRT6 functions as a chromatin regulator safeguarding the balance between pluripotency and differentiation through Tet-mediated production of 5hmC.

Fortier, S., T. MacRae, et al. "Haploinsufficiency screen highlights two distinct groups of ribosomal protein genes essential for embryonic stem cell fate." Proc Natl Acad Sci U S A. 2015 Feb 17;112(7):2127-32. doi: 10.1073/pnas.1418845112. Epub 2015 Feb 2.

In a functional genomics screen of mouse embryonic stem cells (ESCs) with nested hemizygous chromosomal deletions, we reveal that ribosomal protein (RP) genes are the most significant haploinsufficient determinants for embryoid body (EB) formation. Hemizygosity for three RP genes (Rps5, Rps14, or Rps28), distinguished by the proximity of their corresponding protein to the ribosome's mRNA exit site, is associated with the most profound phenotype. This EB phenotype was fully rescued by BAC or cDNA complementation but not by the reduction of p53 levels, although such reduction was effective with most other RP-deleted clones corresponding to non-mRNA exit-site proteins. RNA-sequencing studies further revealed that undifferentiated ESCs hemizygous for Rps5 showed reduced expression levels of several mesoderm-specific genes as compared with wild-type counterparts. Together, these results reveal that RP gene dosage limits the differentiation, not the self-renewal, of mouse ESCs. They also highlight two separate mechanisms underlying this process, one of which is p53 independent.

Galoian, K., A. Qureshi, et al. "Epigenetic regulation of embryonic stem cell marker miR302C in human chondrosarcoma as determinant of antiproliferative activity of proline-rich polypeptide 1." Int J Oncol. 2015 Jun 18. doi: 10.3892/ijo.2015.3054.

Metastatic chondrosarcoma of mesenchymal origin is the second most common bone malignancy and does not respond either to chemotherapy or radiation; therefore, the search for new therapies is relevant and urgent. We described recently that tumor growth inhibiting cytosolic proline-rich polypeptide 1, (PRP-1) significantly upregulated tumor suppressor miRNAs, downregulated onco-miRNAs in human chondrosarcoma JJ012 cell line, compared to chondrocytes culture. In this study we hypothesized the existence and regulation of a functional marker in cancer stem cells, correlated to peptides antiproliferative activity. Experimental results indicated that among significantly downregulated miRNA after PRP-1 treatment was miRNAs 302c*. This miRNA is a part of the cluster miR302367, which is stemness regulator in human embryonic stem cells and in certain tumors, but is not expressed in adult hMSCs and normal tissues. PRP-1 had strong inhibitory effect on viability of chondrosarcoma and multilineage induced multipotent adult cells (embryonic primitive cell type). Unlike chondrosarcoma, in glioblastoma, PRP-1 does not have any inhibitory activity on cell proliferation, because in glioblastoma miR-302-367 cluster plays an opposite role, its expression is sufficient to suppress the stemness inducing properties. The observed correlation between the antiproliferative activity of PRP-1 and its action on downregulation of miR302c explains the peptides opposite effects on the upregulation of proliferation of adult mesenchymal stem cells, and the inhibition of the proliferation of human bone giant-cell tumor stromal cells, reported earlier. PRP-1 substantially downregulated the miR302c targets, the stemness markers Nanog, c-Myc and polycomb protein Bmi-1. miR302c expression is induced by JMJD2-mediated H3K9me2 demethylase activity in its promoter region. JMJD2 was reported to be a positive regulator for Nanog. Our experimental results proved that PRP-1 strongly inhibited H3K9 activity comprised of a pool of JMJD1 and JMJD2. We conclude that inhibition of H3K9 activity by PRP-1 leads to downregulation of miR302c and its targets, defining the PRP-1 antiproliferative role.

Ghanian, M. H., Z. Farzaneh, et al. "Nanotopographical control of human embryonic stem cell differentiation into definitive endoderm." J Biomed Mater Res A. 2015 Apr 22. doi: 10.1002/jbm.a.35483.

Derivation of definitive endoderm (DE) from human embryonic stem cells (hESCs) can address the needs of regenerative medicine for endoderm-derived organs such as the pancreas and liver. Fibrous substrates which topographically recapitulate native extracellular matrix have been known to promote the

stem cell differentiation. However, the optimal fiber diameter remains to be determined for the desired differentiation. Here, we have developed a simple method to precisely fabricate electrospun poly(epsilon-caprolactone) fibers with four distinct average diameters at nano- and microscale levels (200, 500, 800, and 1300 nm). Human ESCs were cultured as clumps or single cells and induced into DE differentiation to determine the optimal topography leading to the promoted differentiation compared with planar culture plates. Gene expression analysis of the DE-induced cells showed significant upregulation of DE-specific genes exclusively on the 200-nm fibers. By Western blot analysis, significant expression of DE-specific proteins was found when hESCs were cultured on the 200 nm substrate as single cells rather than clumps, probably due to more efficient cell-matrix interaction realized by morphological observations of the cell colonies. The results indicated that nanofibrillar substrates, only at ultrathin fiber diameters, provided a better environment for DE differentiation of hESC, which holds great promise in prospective tissue engineering applications. (c) 2015 Wiley Periodicals, Inc. *J Biomed Mater Res Part A*, 2015.

Glass, C., R. Singla, et al. "Mouse embryonic stem cell-derived cardiac myocytes in a cell culture dish." *Methods Mol Biol.* 2015;1299:145-52. doi: [10.1007/978-1-4939-2572-8_11](https://doi.org/10.1007/978-1-4939-2572-8_11).

Embryonic stem (ES) cells are pluripotent stem cells capable of self-renewal and have broad differentiation potential yielding cell types from all three germ layers. In the absence of differentiation inhibitory factors, when cultured in suspension, ES cells spontaneously differentiate and form three-dimensional cell aggregates termed embryoid bodies (EBs). Although various methods exist for the generation of EBs, the hanging drop method offers reproducibility and homogeneity from a predetermined number of ES cells. Herein, we describe the in vitro differentiation of mouse embryonic stem cells into cardiac myocytes using the hanging drop method and immunocytochemistry to identify cardiomyogenic differentiation. In brief, ES cells, placed in droplets on the lid of culture dishes following a 2-day incubation, yield embryoid bodies, which are resuspended and plated. 1-2 weeks following plating of the EBs, spontaneous beating areas can be observed and staining for specific cardiac markers can be achieved.

Grealish, S., A. Heuer, et al. "Monosynaptic Tracing using Modified Rabies Virus Reveals Early and Extensive Circuit Integration of Human Embryonic Stem Cell-Derived Neurons." *Stem Cell Reports.* 2015

Jun 9;4(6):975-83. doi: [10.1016/j.stemcr.2015.04.011](https://doi.org/10.1016/j.stemcr.2015.04.011). Epub 2015 May 21.

Human embryonic stem cell (hESC)-derived dopamine neurons are currently moving toward clinical use for Parkinson's disease (PD). However, the timing and extent at which stem cell-derived neurons functionally integrate into existing host neural circuitry after transplantation remain largely unknown. In this study, we use modified rabies virus to trace afferent and efferent connectivity of transplanted hESC-derived neurons in a rat model of PD and report that grafted human neurons integrate into the host neural circuitry in an unexpectedly rapid and extensive manner. The pattern of connectivity resembled that of local endogenous neurons, while ectopic connections were not detected. Revealing circuit integration of human dopamine neurons substantiates their potential use in clinical trials. Additionally, our data present rabies-based tracing as a valuable and widely applicable tool for analyzing graft connectivity that can easily be adapted to analyze connectivity of a variety of different neuronal sources and subtypes in different disease models.

Hajizadeh-Saffar, E., Y. Tahamtani, et al. "Inducible VEGF expression by human embryonic stem cell-derived mesenchymal stromal cells reduces the minimal islet mass required to reverse diabetes." *Sci Rep.* 2015 Mar 30;5:9322. doi: [10.1038/srep09322](https://doi.org/10.1038/srep09322).

Islet transplantation has been hampered by loss of function due to poor revascularization. We hypothesize that co-transplantation of islets with human embryonic stem cell-derived mesenchymal stromal cells that conditionally overexpress VEGF (hESC-MSC:VEGF) may augment islet revascularization and reduce the minimal islet mass required to reverse diabetes in mice. HESC-MSCs were transduced by recombinant lentiviruses that allowed conditional (Dox-regulated) overexpression of VEGF. HESC-MSC: VEGF were characterized by tube formation assay. After co-transplantation of hESC-MSC:VEGF with murine islets in collagen-fibrin hydrogel in the omental pouch of diabetic nude mice, we measured blood glucose, body weight, glucose tolerance and serum C-peptide. As control, islets were transplanted alone or with non-transduced hESC-MSCs. Next, we compared functional parameters of 400 islets alone versus 200 islets co-transplanted with hESC-MSC:VEGF. As control, 200 islets were transplanted alone. Metabolic function of islets transplanted with hESC-MSC:VEGF significantly improved, accompanied by superior graft revascularization, compared with control groups. Transplantation of 200 islets with hESC-MSC:VEGF showed superior function over 400 islets alone. We conclude that co-transplantation of islets with VEGF-

expressing hESC-MSCs allowed for at least a 50% reduction in minimal islet mass required to reverse diabetes in mice. This approach may contribute to alleviate the need for multiple donor organs per patient.

Hannes, T., M. Wolff, et al. "Electrophysiological characteristics of embryonic stem cell-derived cardiomyocytes are cell line-dependent." Cell Physiol Biochem. 2015;35(1):305-14. doi: [10.1159/000369697](https://doi.org/10.1159/000369697). Epub 2015 Jan 10.

BACKGROUND: Modelling of cardiac development, physiology and pharmacology by differentiation of embryonic stem cells (ESCs) requires comparability of cardiac differentiation between different ESC lines. To investigate whether the outcome of cardiac differentiation is consistent between different ESC lines, we compared electrophysiological properties of ESC-derived cardiomyocytes (ESC-CMs) of different murine ESC lines. **METHODS:** Two wild-type (D3 and R1) and two transgenic ESC lines (D3/aPIG44 and CGR8/AMPIGX-7) were differentiated under identical culture conditions. The transgenic cell lines expressed enhanced green fluorescent protein (eGFP) and puromycin-N-acetyltransferase under control of the cardiac specific alpha-myosin heavy chain (alphaMHC) promoter. Action potentials (APs) were recorded using sharp electrodes and multielectrode arrays in beating clusters of ESC-CMs. **RESULTS:** Spontaneous AP frequency and AP duration (APD) as well as maximal upstroke velocity differed markedly between unpurified CMs of the four ESC lines. APD heterogeneity was negligible in D3/aPIG44, moderate in D3 and R1 and extensive in CGR8/AMPIGX-7. Interspike intervals calculated from long-term recordings showed a high degree of variability within and between recordings in CGR8/AMPIGX-7, but not in D3/aPIG44. Purification of the alphaMHC+ population by puromycin treatment posed only minor changes to APD in D3/aPIG44, but significantly shortened APD in CGR8/AMPIGX-7. **CONCLUSION:** Electrophysiological properties of ESC-CMs are strongly cell line-dependent and can be influenced by purification of cardiomyocytes by antibiotic selection. Thus, conclusions on cardiac development, physiology and pharmacology derived from single stem cell lines have to be interpreted carefully.

Hoeber, J., C. Trolle, et al. "Human Embryonic Stem Cell-Derived Progenitors Assist Functional Sensory Axon Regeneration after Dorsal Root Avulsion Injury." Sci Rep. 2015 Jun 8;5:10666. doi: [10.1038/srep10666](https://doi.org/10.1038/srep10666).

Dorsal root avulsion results in permanent impairment of sensory functions due to disconnection between the peripheral and central nervous system. Improved strategies are therefore needed to reconnect injured sensory neurons with their spinal cord targets in order to achieve functional repair after brachial and lumbosacral plexus avulsion injuries. Here, we show that sensory functions can be restored in the adult mouse if avulsed sensory fibers are bridged with the spinal cord by human neural progenitor (hNP) transplants. Responses to peripheral mechanical sensory stimulation were significantly improved in transplanted animals. Transganglionic tracing showed host sensory axons only in the spinal cord dorsal horn of treated animals. Immunohistochemical analysis confirmed that sensory fibers had grown through the bridge and showed robust survival and differentiation of the transplants. Section of the repaired dorsal roots distal to the transplant completely abolished the behavioral improvement. This demonstrates that hNP transplants promote recovery of sensorimotor functions after dorsal root avulsion, and that these effects are mediated by spinal ingrowth of host sensory axons. These results provide a rationale for the development of novel stem cell-based strategies for functionally useful bridging of the peripheral and central nervous system.

Hogan, M. S., D. E. Parfitt, et al. "Transient pairing of homologous Oct4 alleles accompanies the onset of embryonic stem cell differentiation." Cell Stem Cell. 2015 Mar 5;16(3):275-88. doi: [10.1016/j.stem.2015.02.001](https://doi.org/10.1016/j.stem.2015.02.001).

The relationship between chromatin organization and transcriptional regulation is an area of intense investigation. We characterized the spatial relationships between alleles of the Oct4, Sox2, and Nanog genes in single cells during the earliest stages of mouse embryonic stem cell (ESC) differentiation and during embryonic development. We describe homologous pairing of the Oct4 alleles during ESC differentiation and embryogenesis, and we present evidence that pairing is correlated with the kinetics of ESC differentiation. Importantly, we identify critical DNA elements within the Oct4 promoter/enhancer region that mediate pairing of Oct4 alleles. Finally, we show that mutation of OCT4/SOX2 binding sites within this region abolishes inter-chromosomal interactions and affects accumulation of the repressive H3K9me2 modification at the Oct4 enhancer. Our findings demonstrate that chromatin organization and transcriptional programs are intimately connected in ESCs and that the dynamic positioning of the Oct4 alleles is associated with the transition from pluripotency to lineage specification.

Holzenspies, J., G. Dela Cruz, et al. "Resolving Heterogeneity: Fluorescence-Activated Cell Sorting of Dynamic Cell Populations from Feeder-Free Mouse Embryonic Stem Cell Culture." Methods Mol Biol. 2015 May 31.

Embryonic stem cell (ESC) culture comprises a mixture of cells that are primed to differentiate into different lineages. In conditions where ESCs self-renew, these primed populations continuously interconvert and consequently show highly dynamic coordinated changes in their expression of different sets of pluripotency and differentiation markers. It has become increasingly apparent that this transcriptional heterogeneity is an important characteristic of ESC culture. By sorting for specific populations of ESCs it is possible to enrich for cells with a capacity to colonize the embryo proper or the extra-embryonic lineages such as the descendants of the primitive endoderm or trophoblast. Here, we describe a method of isolating specific sub-sets of ESCs from the pluripotent cells present in in vitro ESC culture using SSEA1 antibody staining in combination with reporter lines and fluorescence activated cell sorting (FACS).

Jacinto, F. V., C. Benner, et al. "The nucleoporin Nup153 regulates embryonic stem cell pluripotency through gene silencing." Genes Dev. 2015 Jun 15;29(12):1224-38. doi: 10.1101/gad.260919.115. Epub 2015 Jun 16.

Nucleoporins (Nups) are a family of proteins best known as the constituent building blocks of nuclear pore complexes (NPCs), membrane-embedded channels that mediate nuclear transport across the nuclear envelope. Recent evidence suggests that several Nups have additional roles in controlling the activation and silencing of developmental genes; however, the mechanistic details of these functions remain poorly understood. Here, we show that depletion of Nup153 in mouse embryonic stem cells (mESCs) causes the derepression of developmental genes and induction of early differentiation. This loss of stem cell identity is not associated with defects in the nuclear import of key pluripotency factors. Rather, Nup153 binds around the transcriptional start site (TSS) of developmental genes and mediates the recruitment of the polycomb-repressive complex 1 (PRC1) to a subset of its target loci. Our results demonstrate a chromatin-associated role of Nup153 in maintaining stem cell pluripotency by functioning in mammalian epigenetic gene silencing.

Jacquet, L., A. Neueder, et al. "Three Huntington's Disease Specific Mutation-Carrying Human Embryonic Stem Cell Lines Have Stable Number of CAG Repeats upon In Vitro Differentiation into Cardiomyocytes." PLoS One. 2015 May

20;10(5):e0126860. doi: 10.1371/journal.pone.0126860. eCollection 2015.

Huntington disease (HD; OMIM 143100), a progressive neurodegenerative disorder, is caused by an expanded trinucleotide CAG (polyQ) motif in the HTT gene. Cardiovascular symptoms, often present in early stage HD patients, are, in general, ascribed to dysautonomia. However, cardio-specific expression of polyQ peptides caused pathological response in murine models, suggesting the presence of a nervous system-independent heart phenotype in HD patients. A positive correlation between the CAG repeat size and severity of symptoms observed in HD patients has also been observed in in vitro HD cellular models. Here, we test the suitability of human embryonic stem cell (hESC) lines carrying HD-specific mutation as in vitro models for understanding molecular mechanisms of cardiac pathology seen in HD patients. We have differentiated three HD-hESC lines into cardiomyocytes and investigated CAG stability up to 60 days after starting differentiation. To assess CAG stability in other tissues, the lines were also subjected to in vivo differentiation into teratomas for 10 weeks. Neither directed differentiation into cardiomyocytes in vitro nor in vivo differentiation into teratomas, rich in immature neuronal tissue, led to an increase in the number of CAG repeats. Although the CAG stability might be cell line-dependent, induced pluripotent stem cells generated from patients with larger numbers of CAG repeats could have an advantage as a research tool for understanding cardiac symptoms of HD patients.

Jenny, R. A., C. Hirst, et al. "Productive Infection of Human Embryonic Stem Cell-Derived NKX2.1+ Respiratory Progenitors With Human Rhinovirus." Stem Cells Transl Med. 2015 Jun;4(6):603-14. doi: 10.5966/sctm.2014-0274. Epub 2015 Apr 14.

: Airway epithelial cells generated from pluripotent stem cells (PSCs) represent a resource for research into a variety of human respiratory conditions, including those resulting from infection with common human pathogens. Using an NKX2.1-GFP reporter human embryonic stem cell line, we developed a serum-free protocol for the generation of NKX2.1(+) endoderm that, when transplanted into immunodeficient mice, matured into respiratory cell types identified by expression of CC10, MUC5AC, and surfactant proteins. Gene profiling experiments indicated that day 10 NKX2.1(+) endoderm expressed markers indicative of early foregut but lacked genes associated with later stages of respiratory epithelial cell differentiation. Nevertheless, NKX2.1(+) endoderm supported the infection and replication of the common respiratory pathogen human rhinovirus HRV1b. Moreover, NKX2.1(+) endoderm upregulated

expression of IL-6, IL-8, and IL-1B in response to infection, a characteristic of human airway epithelial cells. Our experiments provide proof of principle for the use of PSC-derived respiratory epithelial cells in the study of cell-virus interactions. SIGNIFICANCE: This report provides proof-of-principle experiments demonstrating, for the first time, that human respiratory progenitor cells derived from stem cells in the laboratory can be productively infected with human rhinovirus, the predominant cause of the common cold.

Julienne, H., B. Audit, et al. "Embryonic stem cell specific "master" replication origins at the heart of the loss of pluripotency." PLoS Comput Biol. 2015 Feb 6;11(2):e1003969. doi: 10.1371/journal.pcbi.1003969. eCollection 2015 Feb.

Epigenetic regulation of the replication program during mammalian cell differentiation remains poorly understood. We performed an integrative analysis of eleven genome-wide epigenetic profiles at 100 kb resolution of Mean Replication Timing (MRT) data in six human cell lines. Compared to the organization in four chromatin states shared by the five somatic cell lines, embryonic stem cell (ESC) line H1 displays (i) a gene-poor but highly dynamic chromatin state (EC4) associated to histone variant H2AZ rather than a HP1-associated heterochromatin state (C4) and (ii) a mid-S accessible chromatin state with bivalent gene marks instead of a polycomb-repressed heterochromatin state. Plastic MRT regions (less, similar 20% of the genome) are predominantly localized at the borders of U-shaped timing domains. Whereas somatic-specific U-domain borders are gene-dense GC-rich regions, 31.6% of H1-specific U-domain borders are early EC4 regions enriched in pluripotency transcription factors NANOG and OCT4 despite being GC poor and gene deserts. Silencing of these ESC-specific "master" replication initiation zones during differentiation corresponds to a loss of H2AZ and an enrichment in H3K9me3 mark characteristic of late replicating C4 heterochromatin. These results shed a new light on the epigenetically regulated global chromatin reorganization that underlies the loss of pluripotency and lineage commitment.

Jyoti, S. and S. Tandon "Genetic basis for developmental toxicity due to statin intake using embryonic stem cell differentiation model." Hum Exp Toxicol. 2015 Feb 23. pii: 0960327114564795.

The in utero environment is a key factor controlling the fate of the growing embryo. The deleterious effects of statins during the fetal development are still not very well understood. Data from animal studies and retrospective studies

performed in pregnant women give conflicting reports. In this study, using in vitro differentiation model of embryonic stem cells, which mimic the differentiation process of the embryo, we have systematically exposed the cells to lipophilic statins, simvastatin, and atorvastatin at various doses and at critical times during differentiation. The analysis of key genes controlling the differentiation into ecto-, meso- and endodermal lineages was assessed by quantitative polymerase chain reaction. Our results show that genes of the mesodermal lineage were most sensitive to statins, leading to changes in the transcript levels of brachyury, Flk-1, Nkx2.5, and alpha/beta-myosin heavy chain. In addition, changes to endodermal marker alpha-fetoprotein, along with ectodermal Nes and Neurofilament 200 kDa, imply that during early differentiation exposure to these drugs leads to altered signaling, which could translate to the congenital abnormalities seen in the heart and limbs.

Khan, M., E. Nickoloff, et al. "Embryonic Stem Cell-Derived Exosomes Promote Endogenous Repair Mechanisms and Enhance Cardiac Function Following Myocardial Infarction." Circ Res. 2015 Jun 19;117(1):52-64. doi: 10.1161/CIRCRESAHA.117.305990. Epub 2015 Apr 22.

RATIONALE: Embryonic stem cells (ESCs) hold great promise for cardiac regeneration but are susceptible to various concerns. Recently, salutary effects of stem cells have been connected to exosome secretion. ESCs have the ability to produce exosomes, however, their effect in the context of the heart is unknown. OBJECTIVE: Determine the effect of ESC-derived exosome for the repair of ischemic myocardium and whether c-kit(+) cardiac progenitor cells (CPCs) function can be enhanced with ESC exosomes. METHODS AND RESULTS: This study demonstrates that mouse ESC-derived exosomes (mES Ex) possess ability to augment function in infarcted hearts. mES Ex enhanced neovascularization, cardiomyocyte survival, and reduced fibrosis post infarction consistent with resurgence of cardiac proliferative response. Importantly, mES Ex augmented CPC survival, proliferation, and cardiac commitment concurrent with increased c-kit(+) CPCs in vivo 8 weeks after in vivo transfer along with formation of bonafide new cardiomyocytes in the ischemic heart. miRNA array revealed significant enrichment of miR290-295 cluster and particularly miR-294 in ESC exosomes. The underlying basis for the beneficial effect of mES Ex was tied to delivery of ESC specific miR-294 to CPCs promoting increased survival, cell cycle progression, and proliferation. CONCLUSIONS: mES Ex provide

a novel cell-free system that uses the immense regenerative power of ES cells while avoiding the risks associated with direct ES or ES-derived cell transplantation and risk of teratomas. ESC exosomes possess cardiac regeneration ability and modulate both cardiomyocyte and CPC-based repair programs in the heart.

Kiris, E., J. E. Nuss, et al. "Phosphatase Inhibitors Function as Novel, Broad Spectrum Botulinum Neurotoxin Antagonists in Mouse and Human Embryonic Stem Cell-Derived Motor Neuron-Based Assays." *PLoS One*. 2015 Jun 10;10(6):e0129264. doi: 10.1371/journal.pone.0129264. eCollection 2015.

There is an urgent need to develop novel treatments to counter Botulinum neurotoxin (BoNT) poisoning. Currently, the majority of BoNT drug development efforts focus on directly inhibiting the proteolytic components of BoNT, i.e. light chains (LC). Although this is a rational approach, previous research has shown that LCs are extremely difficult drug targets and that inhibiting multi-serotype BoNTs with a single LC inhibitor may not be feasible. An alternative approach would target neuronal pathways involved in intoxication/recovery, rather than the LC itself. Phosphorylation-related mechanisms have been implicated in the intoxication pathway(s) of BoNTs. However, the effects of phosphatase inhibitors upon BoNT activity in the physiological target of BoNTs, i.e. motor neurons, have not been investigated. In this study, a small library of phosphatase inhibitors was screened for BoNT antagonism in the context of mouse embryonic stem cell-derived motor neurons (ES-MNs). Four inhibitors were found to function as BoNT/A antagonists. Subsequently, we confirmed that these inhibitors protect against BoNT/A in a dose-dependent manner in human ES-MNs. Additionally, these compounds provide protection when administered in post-intoxication scenario. Importantly, the inhibitors were also effective against BoNT serotypes B and E. To the best of our knowledge, this is the first study showing phosphatase inhibitors as broad-spectrum BoNT antagonists.

Matveeva, N. M., E. A. Kizilova, et al. "Generation of mouse chimeras with high contribution of tetraploid embryonic stem cells and embryonic stem cell-fibroblast hybrid cells." *Methods Mol Biol*. 2015;1313:61-71. doi: 10.1007/978-1-4939-2703-6_4.

The *in vitro* long-term cultivation of embryonic stem (ES) cells derived from pre-implantation embryos offers the unique possibility of combining ES cells with pre-implantation embryos to generate chimeras, thus facilitating the creation of a bridge between *in vitro* and *in vivo* investigations. Genomic manipulation using ES cells and

homologous recombination is one of the most outstanding scientific achievements, resulting in the generation of animals with desirable genome modifications. As such, the generation of ES cells with different ploidy via cell fusion also deserves much attention because this approach allows for the production of chimeras that contain somatic cells with various ploidy. Therefore, this is a powerful tool that can be used to study the role of polyploidy in the normal development of mammals.

Mehta, R. H. "Sourcing human embryos for embryonic stem cell lines: problems & perspectives." *Indian J Med Res*. 2014 Nov;140 Suppl:S106-11.

The ability to successfully derive human embryonic stem cells (hESC) lines from human embryos following *in vitro* fertilization (IVF) opened up a plethora of potential applications of this technique. These cell lines could have been successfully used to increase our understanding of human developmental biology, transplantation medicine and the emerging science of regenerative medicine. The main source for human embryos has been 'discarded' or 'spare' fresh or frozen human embryos following IVF. It is a common practice to stimulate the ovaries of women undergoing any of the assisted reproductive technologies (ART) and retrieve multiple oocytes which subsequently lead to multiple embryos. Of these, only two or maximum of three embryos are transferred while the rest are cryopreserved as per the decision of the couple. In case a couple does not desire to 'cryopreserve' their embryos then all the embryos remaining following embryo transfer can be considered 'spare' or if a couple is no longer in need of the 'cryopreserved' embryos then these also can be considered as 'spare'. But, the question raised by the ethicists is, "what about 'slightly' over-stimulating a woman to get a few extra eggs and embryos? The decision becomes more difficult when it comes to 'discarded' embryos. As of today, the quality of the embryos is primarily assessed based on morphology and the rate of development mainly judged by single point assessment. Despite many criteria described in the literature, the quality assessment is purely subjective. The question that arises is on the decision of 'discarding' embryos. What would be the criteria for discarding embryos and the potential 'use' of ESC derived from the 'abnormal appearing' embryos? This paper discusses some of the newer methods to procure embryos for the derivation of embryonic stem cell lines which will respect the ethical concerns but still provide the source material.

Melidoni, A. N., M. R. Dyson, et al. "Selection of Antibodies Interfering with Cell Surface Receptor

Signaling Using Embryonic Stem Cell Differentiation." Methods Mol Biol. 2015 Jun 3.

Antibodies able to bind and modify the function of cell surface signaling components in vivo are increasingly being used as therapeutic drugs. The identification of such "functional" antibodies from within large antibody pools is, therefore, the subject of intense research. Here we describe a novel cell-based expression and reporting system for the identification of functional antibodies from antigen-binding populations preselected with phage display. The system involves inducible expression of the antibody gene population from the Rosa-26 locus of embryonic stem (ES) cells, followed by secretion of the antibodies during ES cell differentiation. Target antigens are cell-surface signaling components (receptors or ligands) with a known effect on the direction of cell differentiation (FGFR1 mediating ES cell exit from self renewal in this particular protocol). Therefore, inhibition or activation of these components by functional antibodies in a few elite clones causes a shift in the differentiation outcomes of these clones, leading to their phenotypic selection. Functional antibody genes are then recovered from positive clones and used to produce the purified antibodies, which can be tested for their ability to affect cell fates exogenously. Identified functional antibody genes can be further introduced in different stem cell types. Inducible expression of functional antibodies has a temporally controlled protein-knockdown capability, which can be used to study the unknown role of the signaling pathway in different developmental contexts. Moreover, it provides a means for control of stem cell differentiation with potential in vivo applications.

Menasche, P., V. Vanneaux, et al. "Human embryonic stem cell-derived cardiac progenitors for severe heart failure treatment: first clinical case report." Eur Heart J. 2015 May 19. pii: ehv189.

AIMS: Comparative studies suggest that stem cells committed to a cardiac lineage are more effective for improving heart function than those featuring an extra-cardiac phenotype. We have therefore developed a population of human embryonic stem cell (ESC)-derived cardiac progenitor cells. METHODS AND RESULTS: Undifferentiated human ESCs (I6 line) were amplified and cardiac-committed by exposure to bone morphogenetic protein-2 and a fibroblast growth factor receptor inhibitor. Cells responding to these cardio-instructive cues express the cardiac transcription factor Isl-1 and the stage-specific embryonic antigen SSEA-1 which was then used to purify them by immunomagnetic sorting. The Isl-1+ SSEA-1+ cells were then embedded into a fibrin scaffold which was surgically delivered onto the

infarct area in a 68-year-old patient suffering from severe heart failure [New York Heart Association [NYHA] functional Class III; left ventricular ejection fraction (LVEF): 26%]. A coronary artery bypass was performed concomitantly in a non-infarcted area. The implanted cells featured a high degree of purity (99% were SSEA-1+), had lost the expression of Sox-2 and Nanog, taken as markers for pluripotency, and strongly expressed Isl-1. The intraoperative delivery of the patch was expeditious. The post-operative course was uncomplicated either. After 3 months, the patient is symptomatically improved (NYHA functional Class I; LVEF: 36%) and a new-onset contractility is echocardiographically evident in the previously akinetic cell/patch-treated, non-revascularized area. There have been no complications such as arrhythmias, tumour formation, or immunosuppression-related adverse events. CONCLUSION: This observation demonstrates the feasibility of generating a clinical-grade population of human ESC-derived cardiac progenitors and combining it within a tissue-engineered construct. While any conclusion pertaining to efficacy would be meaningless, the patient's functional outcome yet provides an encouraging hint. Beyond this case, the platform that has been set could be useful for generating different ESC-derived lineage-specific progenies.

Michel-Schmidt, R., C. J. Kirkpatrick, et al. "Effect of LIF-withdrawal on acetylcholine synthesis in the embryonic stem cell line CGR8 is not mediated by STAT3, PI3Ks or cAMP/PKA pathways." Int Immunopharmacol. 2015 Apr 14. pii: S1567-5769(15)00160-5. doi: 10.1016/j.intimp.2015.04.005.

Acetylcholine (ACh) acts as a local cellular signaling molecule and is widely expressed in nature, including mammalian cells and embryonic stem cells. The murine embryonic stem cell line CGR8 synthesizes and releases substantial amounts of ACh. Particularly during early differentiation - a period associated with multiple alterations in geno-/phenotype functions - synthesis and release of ACh are increased by 10-fold. In murine stem cells second messengers of the STAT-3, PI3K and cAMP/PKA pathways are involved in maintaining self-renewal and pluripotency. The present experiments were designed to test whether blockers of these signaling pathways enhance ACh cell content in the presence of LIF, i.e. when CGR8 is pluripotent. NSC74859, an inhibitor of STAT-3, affected neither the proliferation rate nor ACh cell content, whereas the more sensitive STAT-3 inhibitor FLLL31 reduced the proliferation rate and increased ACh cell content by about 3-fold. The PI3K inhibitor LY294002 reduced the proliferation rate but did not modify the ACh cell content, whereas the PKA

inhibitor H89 produced effects comparable to FLLL31. Interestingly, in control experiments a strong inverse correlation was found between cell density and ACh cell content, which could explain the 3-fold increase in the ACh cell content observed in the presence of FLLL31 and H89. Forskolin, a PKA activator, had no effect. In conclusion, it appears unlikely that the 10-fold increase in ACh cell content induced by LIF removal, i.e. during early differentiation, is mediated by second messengers of the STAT-3, PI3K and cAMP/PKA pathways. However, the PI3K pathway appears to be involved in control of the inverse relation between cell density and ACh cell content, because this correlation was significantly attenuated in the presence of LY294002.

Morey, L., A. Santanach, et al. "Pluripotency and epigenetic factors in mouse embryonic stem cell fate regulation." *Mol Cell Biol.* 2015 Jun 1. pii: MCB.00266-15.

Embryonic stem cells (ESCs) are characterized by their ability to self-renew and to differentiate into all cell types of a given organism. Understanding the molecular mechanisms that govern the ESC state is of great interest not only for basic research-for instance, ESCs represent a perfect system to study cellular differentiation in vitro-but also for their potential implications in human health-these mechanisms are likewise involved in cancer progression and could be exploited in regenerative medicine. In this minireview, we focus on the latest insight into the molecular mechanisms mediated by the pluripotency factors as well as their roles during differentiation. We also discuss recent advances on understanding the function of the epigenetic regulators, Polycomb and MLL complexes, in ESC biology.

Movahednia, M. M., F. K. Kidwai, et al. "Differential effects of the extracellular microenvironment on human embryonic stem cell differentiation into keratinocytes and their subsequent replicative life span." *Tissue Eng Part A.* 2015 Apr;21(7-8):1432-43. doi: 10.1089/ten.TEA.2014.0551. Epub 2015 Mar 18.

Culture microenvironment plays a critical role in the propagation and differentiation of human embryonic stem cells (hESCs) and their differentiated progenies. Although high efficiency of hESC differentiation to keratinocytes (hESC-Kert) has been achieved, little is known regarding the effects of early culture microenvironment and pertinent extracellular matrix (ECM) interactions during epidermal commitment on subsequent proliferative capacity of hESC-Kert. The aim of this study is to evaluate the effects of the different ECM microenvironments during hESC differentiation on subsequent replicative

life span of hESC-Kert. In doing so, H1-hESCs were differentiated to keratinocytes (H1-Kert) in two differentiation systems. The first system employed autologous fibroblast feeder support, in which keratinocytes (H1-Kert(ACC)) were derived by coculture of hESCs with hESC-derived fibroblasts (H1-ebFs). The second system employed a novel decellularized matrix from H1-ebFs to create a dermoepidermal junction-like (DEJ) matrix. H1-Kert(AFF) were derived by differentiation of hESCs on the feeder-free system employing the DEJ matrix. Our study indicated that the feeder-free system with the use of DEJ matrix was more efficient in differentiation of hESCs toward epidermal progenitors. However, the feeder-free system was not sufficient to support the subsequent replicative capacity of differentiated keratinocytes. Of note, H1-Kert(AFF) showed limited replicative capacity with reduced telomere length and early cellular senescence. We further showed that the lack of cell-cell interactions during epidermal commitment led to heightened production of TGF-beta1 by hESC-Kert during extended culture, which in turn was responsible for resulting in the limited replicative life span with cellular senescence of hESC-Kert derived under the feeder-free culture system. This study highlights for the first time the importance of the culture microenvironment and cell-ECM interactions during differentiation of hESCs on subsequent replicative life span and cellular senescence of the differentiated keratinocytes, with implications for use of these cells for applications in tissue engineering and regenerative medicine.

Nakhaei-Rad, S., H. Nakhaeizadeh, et al. "The Function of Embryonic Stem Cell-expressed RAS (E-RAS), a Unique RAS Family Member, Correlates with Its Additional Motifs and Its Structural Properties." *J Biol Chem.* 2015 Jun 19;290(25):15892-903. doi: 10.1074/jbc.M115.640607. Epub 2015 May 4.

E-RAS is a member of the RAS family specifically expressed in embryonic stem cells, gastric tumors, and hepatic stellate cells. Unlike classical RAS isoforms (H-, N-, and K-RAS4B), E-RAS has, in addition to striking and remarkable sequence deviations, an extended 38-amino acid-long unique N-terminal region with still unknown functions. We investigated the molecular mechanism of E-RAS regulation and function with respect to its sequence and structural features. We found that N-terminal extension of E-RAS is important for E-RAS signaling activity. E-RAS protein most remarkably revealed a different mode of effector interaction as compared with H-RAS, which correlates with deviations in the effector-binding site of E-RAS. Of all these residues,

tryptophan 79 (arginine 41 in H-RAS), in the interswitch region, modulates the effector selectivity of RAS proteins from H-RAS to E-RAS features.

Nichols, J. and T. Boroviak "Maximizing Clonal Embryonic Stem Cell Derivation by ERK Pathway Inhibition." Methods Mol Biol. 2015 May 31.

Since the development of inhibitor-based defined culture conditions (known as "2i"), multiple clonal embryonic stem cell (ESC) lines can be readily derived from single cells isolated directly from mouse embryos. In addition to providing an efficient means to generate ES cells from compound transgenic or murine disease models on any genetic background, this technology can be used to investigate the process of ESC derivation at both a functional and molecular level. Here, we provide details of the procedure for both maximizing the number of cells in the donor tissue and subsequent effective derivation of multiple clonal ES cell lines.

Orellana, M. D., G. C. De Santis, et al. "Efficient recovery of undifferentiated human embryonic stem cell cryopreserved with hydroxyethyl starch, dimethyl sulphoxide and serum replacement." Cryobiology. 2015 Jan 29. pii: S0011-2240(15)00023-1. doi: 10.1016/j.cryobiol.2015.01.005.

BACKGROUND: The therapeutic use of human embryonic stem cells (hESCs) is dependent on an efficient cryopreservation protocol for long-term storage. The aim of this study was to determine whether the combination of three cryoprotecting reagents using two freezing systems might improve hESC recovery rates with maintenance of hESC pluripotency properties for potential cell therapy application. **METHODS:** Recovery rates of hESC colonies which were frozen in three cryoprotective solutions: Me2SO/HES/SR medium, Defined-medium(R) and Me2SO/SFB in medium solution were evaluated in ultra-slow programmable freezing system (USPF) and a slow-rate freezing system (SRF). The hESC pluripotency properties after freezing-thawing were evaluated. **RESULTS:** We estimated the distribution frequency of survival colonies and observed that independent of the freezing system used (USPF or SRF) the best results were obtained with Me2SO/HES/SR as cryopreservation medium. We showed a significant hESC recovery colonies rate after thawing in Me2SO/HES/SR medium were 3.88 and 2.9 in USPF and SRF, respectively. The recovery colonies rate with Defined-medium(R) were 1.05 and 1.07 however in classical Me2SO medium were 0.5 and 0.86 in USPF and SRF, respectively. We showed significant difference between Me2SO/HES/SR mediumxDefined-medium(R) and between Me2SO/HES/SR mediumxMe2SO medium, for two

cryopreservation systems ($P < 0.05$). **CONCLUSION:** We developed an in house protocol using the combination of Me2SO/HES/SR medium and ultra-slow programmable freezing system which resulted in hESC colonies that remain undifferentiated, maintain their in vitro and in vivo pluripotency properties and genetic stability. This approach may be suitable for cell therapy studies.

Ozeki, N., N. Hase, et al. "Interleukin-1beta-induced autophagy-related gene 5 regulates proliferation of embryonic stem cell-derived odontoblastic cells." PLoS One. 2015 Apr 20;10(4):e0124542. doi: 10.1371/journal.pone.0124542. eCollection 2015.

We previously established a method for the differentiation of induced pluripotent stem cells and embryonic stem cells into alpha2 integrin-positive odontoblast-like cells. We also reported that Wnt5 in response to interleukin (IL)-1beta induces matrix metalloproteinase (MMP)-3-regulated cell proliferation in these cells. Our findings suggest that MMP-3 plays a potentially unique physiological role in the generation of odontoblast-like cells under an inflammatory state. Here, we examined whether up-regulation of autophagy-related gene (Atg) 5 by IL-1beta was mediated by Wnt5 signaling, thus leading to increased proliferation of odontoblast-like cells. IL-1beta increased the mRNA and protein levels of Atg5, microtubule-associated protein 1 light chain (LC3, a mammalian homolog of yeast Atg8) and Atg12. Treatment with siRNAs against Atg5, but not LC3 and Atg12, suppressed the IL-1beta-induced increase in MMP-3 expression and cell proliferation. Our siRNA analyses combined with western blot analysis revealed a unique sequential cascade involving Atg5, Wnt5a and MMP-3, which resulted in the potent increase in odontoblastic cell proliferation. These results demonstrate the unique involvement of Atg5 in IL-1beta-induced proliferation of embryonic stem cell-derived odontoblast-like cells.

Pearson, S., S. Cuvertino, et al. "In vivo repopulating activity emerges at the onset of hematopoietic specification during embryonic stem cell differentiation." Stem Cell Reports. 2015 Mar 10;4(3):431-44. doi: 10.1016/j.stemcr.2015.01.003. Epub 2015 Feb 5.

The generation of in vivo repopulating hematopoietic cells from in vitro differentiating embryonic stem cells has remained a long-standing challenge. To date, hematopoietic engraftment has mostly been achieved through the enforced expression of ectopic transcription factors. Here, we describe serum-free culture conditions that allow the generation of in vivo repopulating hematopoietic cells in the absence of ectopically expressed factors. We show

that repopulating activity arises immediately upon the commitment of mesodermal precursors to the blood program, within the first wave of hematopoietic specification. We establish that the formation of these progenitors is extremely transient and exquisitely sensitive to the cytokine milieu. Our findings define the precise differentiating stage at which hematopoietic repopulating activity first appears in vitro, and suggest that during embryonic stem cell differentiation, all hematopoietic programs are unraveled simultaneously from the mesoderm in the absence of cues that restrict the coordinated emergence of each lineage as is normally observed during embryogenesis.

Piao, J., T. Major, et al. "Human embryonic stem cell-derived oligodendrocyte progenitors remyelinate the brain and rescue behavioral deficits following radiation." *Cell Stem Cell*. 2015 Feb 5;16(2):198-210. doi: [10.1016/j.stem.2015.01.004](https://doi.org/10.1016/j.stem.2015.01.004).

Radiation therapy to the brain is a powerful tool in the management of many cancers, but it is associated with significant and irreversible long-term side effects, including cognitive decline and impairment of motor coordination. Depletion of oligodendrocyte progenitors and demyelination are major pathological features that are particularly pronounced in younger individuals and severely limit therapeutic options. Here we tested whether human ESC-derived oligodendrocytes can functionally remyelinate the irradiated brain using a rat model. We demonstrate the efficient derivation and prospective isolation of human oligodendrocyte progenitors, which, upon transplantation, migrate throughout the major white matter tracts resulting in both structural and functional repair. Behavioral testing showed complete recovery of cognitive function while additional recovery from motor deficits required concomitant transplantation into the cerebellum. The ability to repair radiation-induced damage to the brain could dramatically improve the outlook for cancer survivors and enable more effective use of radiation therapies, especially in children.

Piatti, P., C. Y. Lim, et al. "Embryonic stem cell differentiation requires full length Chd1." *Sci Rep*. 2015 Jan 26;5:8007. doi: [10.1038/srep08007](https://doi.org/10.1038/srep08007).

The modulation of chromatin dynamics by ATP-dependent chromatin remodeling factors has been recognized as an important mechanism to regulate the balancing of self-renewal and pluripotency in embryonic stem cells (ESCs). Here we have studied the effects of a partial deletion of the gene encoding the chromatin remodeling factor Chd1 that generates an N-terminally truncated version of Chd1 in mouse ESCs in vitro as well as in vivo. We

found that a previously uncharacterized serine-rich region (SRR) at the N-terminus is not required for chromatin assembly activity of Chd1 but that it is subject to phosphorylation. Expression of Chd1 lacking this region in ESCs resulted in aberrant differentiation properties of these cells. The self-renewal capacity and ESC chromatin structure, however, were not affected. Notably, we found that newly established ESCs derived from Chd1(Delta2/Delta2) mutant mice exhibited similar differentiation defects as in vitro generated mutant ESCs, even though the N-terminal truncation of Chd1 was fully compatible with embryogenesis and post-natal life in the mouse. These results underscore the importance of Chd1 for the regulation of pluripotency in ESCs and provide evidence for a hitherto unrecognized critical role of the phosphorylated N-terminal SRR for full functionality of Chd1.

Ramos-Ibeas, P., E. Pericuesta, et al. "Characterisation of the deleted in azoospermia like (Dazl)-green fluorescent protein mouse model generated by a two-step embryonic stem cell-based strategy to identify pluripotent and germ cells." *Reprod Fertil Dev*. 2015 May 6. doi: [10.1071/RD14253](https://doi.org/10.1071/RD14253).

The deleted in azoospermia like (Dazl) gene is preferentially expressed in germ cells; however, recent studies indicate that it may have pluripotency-related functions. We generated Dazl-green fluorescent protein (GFP) transgenic mice and assayed the ability of Dazl-driven GFP to mark preimplantation embryo development, fetal, neonatal and adult tissues, and in vitro differentiation from embryonic stem cells (ESCs) to embryoid bodies (EBs) and to primordial germ cell (PGC)-like cells. The Dazl-GFP mice were generated by a two-step ESC-based strategy, which enabled primary and secondary screening of stably transfected clones before embryo injection. During preimplantation embryo stages, GFP was detected from the zygote to blastocyst stage. At Embryonic Day (E) 12.5, GFP was expressed in gonadal ridges and in neonatal gonads of both sexes. In adult mice, GFP expression was found during spermatogenesis from spermatogonia to elongating spermatids and in the cytoplasm of oocytes. However, GFP mRNA was also detected in other tissues harbouring multipotent cells, such as the intestine and bone marrow. Fluorescence was maintained along in vitro Dazl-GFP ESC differentiation to EBs, and in PGC-like cells. In addition to its largely known function in germ cell development, Dazl could have an additional role in pluripotency, supporting these transgenic mice as a valuable tool for the prospective identification of stem cells from several tissues.

Robbez-Masson, L. and H. M. Rowe "Retrotransposons shape species-specific embryonic stem cell gene expression." Retrovirology. 2015 May 29;12:45. doi: 10.1186/s12977-015-0173-5.

Over half of our genome is composed of retrotransposons, which are mobile elements that can readily amplify their copy number by replicating through an RNA intermediate. Most of these elements are no longer mobile but still contain regulatory sequences that can serve as promoters, enhancers or repressors for cellular genes. Despite dominating our genetic content, little is known about the precise functions of retrotransposons, which include both endogenous retroviruses (ERVs) and non-LTR elements like long interspersed nuclear element 1 (LINE-1). However, a few recent cutting-edge publications have illustrated how retrotransposons shape species-specific stem cell gene expression by two opposing mechanisms, involving their recruitment of stem cell-enriched transcription factors (TFs): firstly, they can activate expression of genes linked to naive pluripotency, and secondly, they can induce repression of proximal genes. The paradox that different retrotransposons are active or silent in embryonic stem cells (ESCs) can be explained by differences between retrotransposon families, between individual copies within the same family, and between subpopulations of ESCs. Since they have coevolved with their host genomes, some of them have been co-opted to perform species-specific beneficial functions, while others have been implicated in genetic disease. In this review, we will discuss retrotransposon functions in ESCs, focusing on recent mechanistic advances of how HERV-H has been adopted to preserve human naive pluripotency and how particular LINE-1, SVA and ERV family members recruit species-specific transcriptional repressors. This review highlights the fine balance between activation and repression of retrotransposons that exists to harness their ability to drive evolution, while minimizing the risk they pose to genome integrity.

Sarkar, P., S. M. Randall, et al. "Activin/nodal signaling switches the terminal fate of human embryonic stem cell-derived trophoblasts." J Biol Chem. 2015 Apr 3;290(14):8834-48. doi: 10.1074/jbc.M114.620641. Epub 2015 Feb 10.

Human embryonic stem cells (hESCs) have been routinely treated with bone morphogenetic protein and/or inhibitors of activin/nodal signaling to obtain cells that express trophoblast markers. Trophoblasts can terminally differentiate to either extravillous trophoblasts or syncytiotrophoblasts. The signaling pathways that govern the terminal fate of these trophoblasts are not understood. We show that activin/nodal signaling switches the terminal fate of

these hESC-derived trophoblasts. Inhibition of activin/nodal signaling leads to formation of extravillous trophoblast, whereas loss of activin/nodal inhibition leads to the formation of syncytiotrophoblasts. Also, the ability of hESCs to form bona fide trophoblasts has been intensely debated. We have examined hESC-derived trophoblasts in the light of stringent criteria that were proposed recently, such as hypomethylation of the ELF5-2b promoter region and down-regulation of HLA class I antigens. We report that trophoblasts that possess these properties can indeed be obtained from hESCs.

Schmitt, J., S. Eckardt, et al. "Human parthenogenetic embryonic stem cell-derived neural stem cells express HLA-G and show unique resistance to NK cell-mediated killing." Mol Med. 2015 Mar 23. doi: 10.2119/molmed.2014.00188.

Parent-of-origin imprints have been implicated in the regulation of neural differentiation and brain development. Previously we have shown that, despite the lack of a paternal genome, human parthenogenetic (PG) embryonic stem cells (hESCs) can form proliferating neural stem cells (NSCs) that are capable of differentiation into physiologically functional neurons while maintaining allele-specific expression of imprinted genes. Since normal hESC-derived NSCs (N NSCs) are targeted by immune cells, we characterized the immunogenicity of PG NSCs. Flow cytometry and immunocytochemistry revealed that both N NSCs and PG NSCs exhibited surface expression of human leukocyte antigen (HLA)-class I but not HLA-DR molecules. Functional analyses using an in vitro mixed lymphocyte reaction assay resulted in less proliferation of peripheral blood mononuclear cells with PG compared to N NSCs. In addition, natural killer cells cytolysed PG less than N NSCs. At a molecular level, expression analyses of immune regulatory factors revealed higher HLA-G levels in PG compared to N NSCs. In line with this finding, MIR152, which represses HLA-G expression, is less transcribed in PG compared to N cells. Blockage of HLA-G receptors ILT2 and KIR2DL4 on NK cells increased cytolysis of PG NSCs. Together this indicates that PG NSCs have unique immunological properties due to elevated HLA-G expression.

Schulpen, S. H., J. L. Pennings, et al. "Gene Expression Regulation and Pathway Analysis After Valproic Acid and Carbamazepine Exposure in a Human Embryonic Stem Cell-Based Neurodevelopmental Toxicity Assay." Toxicol Sci. 2015 May 15. pii: kfv094.

Differentiating pluripotent stem cells in vitro have proven useful for the study of developmental toxicity. Here, we studied the effects of anticonvulsant drug exposure in a human embryonic stem cell (hESC)-based neurodevelopmental toxicity test (hESTn). During neural differentiation the cells were exposed, for either 1 or 7 days, to noncytotoxic concentration ranges of valproic acid (VPA) or carbamazepine (CBZ), antiepileptic drugs known to cause neurodevelopmental toxicity. The effects observed on gene expression and correlated processes and pathways were in line with processes associated with neural development and pharmaceutical mode of action. In general, VPA showed a higher number of genes and molecular pathways affected than CBZ. The response kinetics differed between both compounds, with CBZ showing higher response magnitudes at day 1, versus VPA at day 7. With this study, we demonstrated the potential and biological relevance of the application of this hESC-based differentiation assay in combination with transcriptomics, as a tool to study neurodevelopmental toxicity.

Sikorski, D. J., N. J. Caron, et al. "Clonal analysis of individual human embryonic stem cell differentiation patterns in microfluidic cultures." *Biotechnol J.* 2015 Jun 9. doi: 10.1002/biot.201500035.

Heterogeneity in the clonal outputs of individual human embryonic stem cells (hESCs) confounds analysis of their properties in studies of bulk populations and how to manipulate them for clinical applications. To circumvent this problem we developed a microfluidic device that supports the robust generation of colonies derived from single ESCs. This microfluidic system contains 160 individually addressable chambers equipped for perfusion culture of individual hESCs that could be shown to match the growth rates, marker expression and colony morphologies obtained in conventional cultures. Use of this microfluidic device to analyze the clonal growth kinetics of multiple individual hESCs induced to differentiation revealed variable shifts in the growth rate, area per cell and expression of OCT4 in the progeny of individual hESCs. Interestingly, low OCT4 expression, a slower growth rate and low nuclear to cytoplasmic ratios were found to be correlated responses. This study demonstrates how microfluidic systems can be used to enable large scale live-cell imaging of isolated hESCs exposed to changing culture conditions, to examine how different aspects of their variable responses are correlated.

Siriboon, C., Y. H. Lin, et al. "Putative porcine embryonic stem cell lines derived from aggregated four-celled cloned embryos produced by oocyte bisection cloning." *PLoS One.* 2015 Feb

13;10(2):e0118165. doi: 10.1371/journal.pone.0118165. eCollection 2015.

We attempted to isolate ES cell lines using inner cell masses from high-quality cloned porcine blastocysts. After being seeded onto feeders, embryos had better ($P < 0.05$) attachment, outgrowth formation and primary colonization in both 2x and 3x aggregated cloned embryos (62.8, 42.6 and 12.8% vs. 76.2, 55.2 and 26.2%, respectively) compared to the non-aggregated group (41.6, 23.4 and 3.9%). Effects of feeder types (STO vs. MEF) and serum sources (FBS vs. KSR) on extraction of cloned embryo-derived porcine ES cells were examined. More (17.1%) ntES cell lines over Passage 3 were generated in the MEF/KSR group. However, ntES cells cultured in KSR-supplemented medium had a low proliferation rate with defective morphology, and eventually underwent differentiation or apoptosis subsequently. Approximately 26.1, 22.7 and 35.7% of primary colonies were formed after plating embryos in DMEM, DMEM/F12 and alpha-MEM media, respectively. Survival rates of ntES cells cultured in alpha-MEM, DMEM and DMEM/F12 were 16.7, 4.3 and 6.8%, respectively ($P > 0.05$). We further examined the beneficial effect of TSA treatment of 3x aggregated cloned embryos on establishment of ntES cell lines. Primary colony numbers and survival rates of ntES cells beyond passage 3 were higher ($P < 0.05$) in those derived from TSA-treated 3x blastocysts (36.7 and 26.7%) than from the non-treated aggregated group (23.1 and 11.5%).

Sivaraman, M. A. and S. N. Noor "Human Embryonic Stem Cell Research: Ethical Views of Buddhist, Hindu and Catholic Leaders in Malaysia." *Sci Eng Ethics.* 2015 Jun 7.

Embryonic Stem Cell Research (ESCR) raises ethical issues. In the process of research, embryos may be destroyed and, to some, such an act entails the 'killing of human life'. Past studies have sought the views of scientists and the general public on the ethics of ESCR. This study, however, explores multi-faith ethical viewpoints, in particular, those of Buddhists, Hindus and Catholics in Malaysia, on ESCR. Responses were gathered via semi-structured, face-to-face interviews. Three main ethical quandaries emerged from the data: (1) sanctity of life, (2) do no harm, and (3) 'intention' of the research. Concerns regarding the sanctity of life are directed at particular research protocols which interfere with religious notions of human ensoulment and early consciousness. The principle of 'do no harm' which is closely related to ahimsa prohibits all acts of violence. Responses obtained indicate that respondents either discourage research that inflicts harm on living entities or allow ESCR with reservations. 'Intention' of

the research seems to be an interesting and viable rationale that would permit ESCR for the Buddhists and Hindus. Research that is intended for the purpose of alleviating human suffering is seen as being ethical. This study also notes that Catholics oppose ESCR on the basis of the inviolability of human life.

Skarnes, W. C. "Is mouse embryonic stem cell technology obsolete?" Genome Biol. 2015 May 27;16:109. doi: 10.1186/s13059-015-0673-6.

Injection of recombinant Cas9 protein and synthetic guide RNAs into mouse zygotes has been shown to facilitate gene disruption and knock-ins using the CRISPR system. These technologies may soon displace genetic modification using embryonic stem cells.

Song, W. K., K. M. Park, et al. "Treatment of macular degeneration using embryonic stem cell-derived retinal pigment epithelium: preliminary results in asian patients." Stem Cell Reports. 2015 May 12;4(5):860-72. doi: 10.1016/j.stemcr.2015.04.005. Epub 2015 Apr 30.

Embryonic stem cells hold great promise for various diseases because of their unlimited capacity for self-renewal and ability to differentiate into any cell type in the body. However, despite over 3 decades of research, there have been no reports on the safety and potential efficacy of pluripotent stem cell progeny in Asian patients with any disease. Here, we report the safety and tolerability of subretinal transplantation of human embryonic-stem-cell (hESC)-derived retinal pigment epithelium in four Asian patients: two with dry age-related macular degeneration and two with Stargardt macular dystrophy. They were followed for 1 year. There was no evidence of adverse proliferation, tumorigenicity, ectopic tissue formation, or other serious safety issues related to the transplanted cells. Visual acuity improved 9-19 letters in three patients and remained stable (+1 letter) in one patient. The results confirmed that hESC-derived cells could serve as a potentially safe new source for regenerative medicine.

Sorkio, A., P. J. Porter, et al. "Surface modified biodegradable electrospun membranes as a carrier for human embryonic stem cell derived retinal pigment epithelial cells." Tissue Eng Part A. 2015 May 6.

Human embryonic stem cell derived retinal pigment epithelial (hESC-RPE) cells are currently undergoing clinical trials to treat retinal degenerative diseases. Transplantation of hESC-RPE cells in conjunction with a supportive biomaterial carrier hold great potential as a future treatment for retinal degeneration. However, there has been no such biodegradable material which could support the

growth and maturation of hESC-RPE cells so far. The primary aim of this work was to create a thin porous poly (L-lactide-co-caprolactone) (PLCL) membrane that could promote attachment, proliferation and maturation of the hESC-RPE cells in serum-free culture conditions. The PLCL membranes were modified by atmospheric pressure plasma processing and coated with collagen IV to enhance cell growth and maturation. Permeability of the membranes was analysed with Ussing chamber system. Analysis with scanning electron microscopy, contact angle measurement, atomic force microscopy and X-ray photoelectron spectroscopy demonstrated that plasma surface treatment augments the surface properties of the membrane, which enhances the binding and conformation of the protein. Cell proliferation assays, RT-PCR, indirect immunofluorescence staining, trans-epithelial electrical resistance measurements as well as in vitro phagocytosis assay clearly demonstrated that the plasma treated PLCL membranes supported the adherence, proliferation, maturation and functionality of hESC-RPE cells in serum-free culture conditions. Here, we report for the first time, how PLCL membranes can be modified with atmospheric pressure plasma processing to enable the formation of a functional hESC-RPE monolayer on a porous biodegradable substrate, which have a potential as a tissue engineered construct for regenerative retinal repair applications.

Tosca, L., O. Feraud, et al. "Genomic instability of human embryonic stem cell lines using different passaging culture methods." Mol Cytogenet. 2015 Apr 23;8:30. doi: 10.1186/s13039-015-0133-8. eCollection 2015.

BACKGROUND: Human embryonic stem cells exhibit genomic instability that can be related to culture duration or to the passaging methods used for cell dissociation. In order to study the impact of cell dissociation techniques on human embryonic stem cells genomic instability, we cultured H1 and H9 human embryonic stem cells lines using mechanical/manual or enzymatic/collagenase-IV dissociation methods. Genomic instability was evaluated at early (<p60) and late (>p60) passages by using oligonucleotide based array-comparative genomic hybridization 105 K with a mean resolution of 50 Kb. **RESULTS:** DNA variations were mainly located on subtelomeric and pericentromeric regions with sizes <100 Kb. In this study, 9 recurrent genomic variations were acquired during culture including the well known duplication 20q11.21. When comparing cell dissociation methods, we found no significant differences between DNA variations number and size, DNA gain or DNA loss frequencies, homozygous loss frequencies and no significant difference on the

content of genes involved in development, cell cycle tumorigenesis and syndrome disease. In addition, we have never found any malignant tissue in 4 different teratoma representative of the two independent stem cell lines. CONCLUSIONS: These results show that the occurrence of genomic instability in human embryonic stem cells is similar using mechanical or collagenase IV-based enzymatic cell culture dissociation methods. All the observed genomic variations have no impact on the development of malignancy.

Vairo, L., E. Medei, et al. "Functional properties of a Brazilian derived mouse embryonic stem cell line." *An Acad Bras Cienc.* 2015 Mar;87(1):275-88. doi: [10.1590/0001-3765201520140474](https://doi.org/10.1590/0001-3765201520140474). Epub 2015 Mar 6.

Pluripotent mouse embryonic stem cells (mESC) are cell lines derived from the inner cell mass of blastocyst-stage early mammalian embryos. Since ion channel modulation has been reported to interfere with both growth and differentiation process in mouse and human ESC it is important to characterize the electrophysiological properties of newly generated mESC and compare them to other lines. In this work, we studied the intercellular communication by way of gap junctions in a Brazilian derived mESC (USP-1, generated by Dr. Lygia Pereira's group) and characterized its electrophysiological properties. We used immunofluorescence and RT-PCR to reveal the presence of connexin 43 (Cx43), pluripotency markers and ion channels. Using a co-culture of neonatal mouse cardiomyocytes with mESC, where the heart cells expressed the enhanced Green Fluorescent Protein, we performed dye injections to assess functional coupling between the two cell types observing dye diffusion. The patch-clamp study showed outward currents identified as two types of potassium currents, transient outward potassium current (I_{to}) and delayed rectifier outward potassium current (I_{ks}), by use of specific drug blockage. Calcium or sodium currents in undifferentiated mESC were not identified. We conclude that USP-1 mESC has functional Cx43 channels establishing intercellular communication among themselves and with cardiomyocytes and has a similar electrophysiological profile compared to other mESC cell lines.

Vakili, K., A. M. McGahan, et al. "Progress in human embryonic stem cell research in the United States between 2001 and 2010." *PLoS One.* 2015 Mar 26;10(3):e0120052. doi: [10.1371/journal.pone.0120052](https://doi.org/10.1371/journal.pone.0120052). eCollection 2015.

On August 9th, 2001, the federal government of the United States announced a policy restricting federal funds available for research on human embryonic stem cell (hESCs) out of concern for the

"vast ethical mine fields" associated with the creation of embryos for research purposes. Until the policy was repealed on March 9th, 2009, no U.S. federal funds were available for research on hESCs extracted after August 9, 2001, and only limited federal funds were available for research on a subset of hESC lines that had previously been extracted. This paper analyzes how the 2001 U.S. federal funding restrictions influenced the quantity and geography of peer-reviewed journal publications on hESC. The primary finding is that the 2001 policy did not have a significant aggregate effect on hESC research in the U.S. After a brief lag in early 2000s, U.S. hESC research maintained pace with other areas of stem cell and genetic research. The policy had several other consequences. First, it was tied to increased hESC research funding within the U.S. at the state level, leading to concentration of related activities in a relatively small number of states. Second, it stimulated increased collaborative research between US-based scientists and those in countries with flexible policies toward hESC research (including Canada, the U.K., Israel, China, Spain, and South Korea). Third, it encouraged independent hESC research in countries without restrictions.

Van der Jeught, M., J. Taelman, et al. "Application Of Small Molecules Favoring Naive Pluripotency during Human Embryonic Stem Cell Derivation." *Cell Reprogram.* 2015 Jun;17(3):170-80. doi: [10.1089/cell.2014.0085](https://doi.org/10.1089/cell.2014.0085).

In mice, inhibition of both the fibroblast growth factor (FGF) mitogen-activated protein kinase kinase/extracellular-signal regulated kinase (MEK/Erk) and the Wnt signaling inhibitor glycogen synthase-3beta (GSK3beta) enables the derivation of mouse embryonic stem cells (mESCs) from nonpermissive strains in the presence of leukemia inhibitory factor (LIF). Whereas mESCs are in an uncommitted naive state, human embryonic stem cells (hESCs) represent a more advanced state, denoted as primed pluripotency. This burdens hESCs with a series of characteristics, which, in contrast to naive ESCs, makes them not ideal for key applications such as cell-based clinical therapies and human disease modeling. In this study, different small molecule combinations were applied during human ESC derivation. Hereby, we aimed to sustain the naive pluripotent state, by interfering with various key signaling pathways. First, we tested several combinations on existing, 2i (PD0325901 and CHIR99021)-derived mESCs. All combinations were shown to be equally adequate to sustain the expression of naive pluripotency markers. Second, these conditions were tested during hESC derivation. Overall, the best results were observed in the presence

of medium supplemented with 2i, LIF, and the noncanonical Wnt signaling agonist Wnt5A, alone and combined with epinephrine. In these conditions, outgrowths repeatedly showed an ESC progenitor-like morphology, starting from day 3. Culturing these "progenitor cells" did not result in stable, naive hESC lines in the current conditions. Although Wnt5A could not promote naive hESC derivation, we found that it was sustaining the conversion of established hESCs toward a more naive state. Future work should aim to distinct the effects of the various culture formulations, including our Wnt5A-supplemented medium, reported to promote stable naive pluripotency in hESCs.

Weber, M., D. Schlembach, et al. "PP044. Differential expression of trophoblast-, endothelial- and embryonic stem cell-associated transcription factors in 1st trimester, and in 3rd trimester preeclampsia and intrauterine growth restriction." *Pregnancy Hypertens.* 2013 Apr;3(2):82. doi: 10.1016/j.preghy.2013.04.071. Epub 2013 Jun 6.

OBJECTIVES: Trophoblast progenitor cells express stem cells markers (SCM) to maintain the proliferative characteristic of stem cells. Beyond blastocyst stage or in preeclampsia (PE) or intrauterine growth restriction (IUGR) little is known about expression of SCMs. We examined the expression of trophoblast and other SCMs in 1st and 3rd trimester placenta and in preeclampsia (PE) and intrauterine growth restriction (IUGR) in order to discriminate if these markers might be involved in progenitor cell functions. **METHODS:** 8 samples each of 1st trimester placentae (elective abortions), 3rd trimester IUGR, PE and control (normal term pregnancy placentae) were stained by immunoperoxidase to detect the SCMs: CDX2 (trophoblast SCM), SOX2, NANOG and OCT4A (embryonic SCMs) and NOTCH1 (endothelial SCM). **RESULTS:** In 1st trimester all SCM were detected, expressed homogenous in syncytio- and cytotrophoblast, and grow increasingly mosaic-like towards the end of 1st trimester. These signals are lost or diminished in 3rd trimester, whereby the syncytiotrophoblast loses these signals first. NOTCH1, however, remains highly expressed in all trophoblast subtypes of both IUGR and PE pregnancies. **CONCLUSION:** Both embryonic and trophoblast SCMs are expressed in 1st trimester trophoblast and appear most vivid among the villous trophoblast of very early pregnancy. Loss of stem cell transcription factor expression in term placentae indicates temporal regulation, and a so far unknown specific function.

Werneburg, S., F. F. Buettner, et al. "Polysialic acid modification of the synaptic cell adhesion molecule

SynCAM 1 in human embryonic stem cell-derived oligodendrocyte precursor cells." *Stem Cell Res.* 2015 May;14(3):339-46. doi: 10.1016/j.scr.2015.03.001. Epub 2015 Mar 21.

Oligodendrocyte precursor cells (OPCs) are the progenitors of myelinating oligodendrocytes in brain development and repair. Successful myelination depends on the control of adhesiveness during OPC migration and axon contact formation. The decoration of cell surface proteins with the glycan polysialic acid (polySia) is a key regulatory element of OPC interactions during development and under pathological conditions. By far the major protein carrier of polySia is the neural cell adhesion molecule NCAM, but recently, polysialylation of the synaptic cell adhesion molecule SynCAM 1 has been detected in the developing mouse brain. In mice, polySia-SynCAM 1 is associated with cells expressing NG2, a marker of a heterogeneous precursor cell population, which is the primary source for oligodendrocytes in development and myelin repair but can also give rise to astrocytes and possibly neurons. It is not yet clear if polySia-SynCAM 1 is expressed by OPCs and its occurrence in humans is elusive. By generating uniform human embryonic stem cell-derived OPC cultures, we demonstrate that polySia is present on human OPCs but down-regulated during differentiation into myelin basic protein-positive oligodendrocytes. PolySia on NCAM resides on the isoforms NCAM-180 and NCAM-140, and SynCAM 1 is identified as a novel polySia acceptor in human OPCs.

Wettstein, R., M. Bodak, et al. "Generation of a Knockout Mouse Embryonic Stem Cell Line Using a Paired CRISPR/Cas9 Genome Engineering Tool." *Methods Mol Biol.* 2015 Mar 12.

CRISPR/Cas9, originally discovered as a bacterial immune system, has recently been engineered into the latest tool to successfully introduce site-specific mutations in a variety of different organisms. Composed only of the Cas9 protein as well as one engineered guide RNA for its functionality, this system is much less complex in its setup and easier to handle than other guided nucleases such as Zinc-finger nucleases or TALENs. Here, we describe the simultaneous transfection of two paired CRISPR sgRNAs-Cas9 plasmids, in mouse embryonic stem cells (mESCs), resulting in the knockout of the selected target gene. Together with a four primer-evaluation system, it poses an efficient way to generate new independent knockout mouse embryonic stem cell lines.

Zarei Fard, N., T. Talaei-Khozani, et al. "Comparison of cell viability and embryoid body size of two

embryonic stem cell lines after different exposure times to bone morphogenetic protein 4." *Iran J Med Sci.* 2015 Mar;40(2):110-7.

BACKGROUND: Activation of bone morphogenetic protein 4 (BMP4) signaling pathway in embryonic stem (ES) cells plays an important role in controlling cell proliferation, differentiation, and apoptosis. Adverse effects of BMP4 occur in a time dependent manner; however, little is known about the effect of different time exposure of this growth factor on cell number in culture media. In this study, we investigated the role of two different exposure times to BMP4 in cell viability, embryoid body (EB), size, and cavitation of ES cells. **METHODS:** Embryonic stem cells (R1 and B1 lines) were released from the feeder cell layers and were cultured using EBs protocol by using the hanging drop method and monolayer culture system. The cells were cultured for 5 days with 100 ng/mL BMP4 from the beginning (++)BMP4) or after 48 h (+BMP4) of culture and their cell number were counted by trypan blue staining. The data were analyzed using non-parametric two-tailed Mann-Whitney test. $P < 0.05$ was considered as significant. **RESULTS:** In EB culture protocol, cell number significantly decreased in +BMP4 culture condition with greater cavity size compared to the ++BMP4 condition at day 5 ($P = 0.009$). In contrast, in monolayer culture system, there was no significant difference in the cell number between all groups ($P = 0.91$). **CONCLUSION:** The results suggest that short-term exposure of BMP4 is required to promote cavitation in EBs according to lower cell number in +BMP4 condition. Different cell lines showed different behavior in cavitation formation.

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References

- Alajem, A., A. Biran, et al. "Differential association of chromatin proteins identifies BAF60a/SMARCD1 as a regulator of embryonic stem cell differentiation." *Cell Rep.* 2015 Mar 31;10(12):2019-31. doi: 10.1016/j.celrep.2015.02.064. Epub 2015 Mar 26.
- Amer, L. D., A. Holtzinger, et al. "Enzymatically degradable poly(ethylene glycol) hydrogels for the 3D culture and release of human embryonic stem cell derived pancreatic precursor cell aggregates." *Acta Biomater.* 2015 Aug;22:103-10. doi: 10.1016/j.actbio.2015.04.013. Epub 2015 Apr 22.
- Bertero, A., P. Madrigal, et al. "Activin/nodal signaling and NANOG orchestrate human embryonic stem cell fate decisions by controlling the H3K4me3 chromatin mark." *Genes Dev.* 2015 Apr 1;29(7):702-17. doi: 10.1101/gad.255984.114. Epub 2015 Mar 24.
- Bruin, J. E., N. Saber, et al. "Treating diet-induced diabetes and obesity with human embryonic stem cell-derived pancreatic progenitor cells and antidiabetic drugs." *Stem Cell Reports.* 2015 Apr 14;4(4):605-20. doi: 10.1016/j.stemcr.2015.02.011. Epub 2015 Mar 19.
- Corradi, S., E. Dakou, et al. "Morphological observation of embryoid bodies completes the in vitro evaluation of nanomaterial embryotoxicity in the embryonic stem cell test (EST)." *Toxicol In Vitro.* 2015 Jun 17. pii: S0887-2333(15)00151-4. doi: 10.1016/j.tiv.2015.06.015.
- Ehnes, D. D., R. M. Geransar, et al. "Exogenous nitric oxide enhances calcification in embryonic stem cell-derived osteogenic cultures." *Differentiation.* 2015 Mar-Apr;89(3-4):97-103. doi: 10.1016/j.diff.2015.02.001. Epub 2015 Apr 27.
- Elert-Dobkowska, E., J. C. Hennings, et al. "Multiplex ligation-dependent probe amplification for identification of correctly targeted murine embryonic stem cell clones." *Anal Biochem.* 2015 Apr 1;474:35-7. doi: 10.1016/j.ab.2015.01.007. Epub 2015 Jan 20.
- Etchegaray, J. P., L. Chavez, et al. "The histone deacetylase SIRT6 controls embryonic stem cell fate via TET-mediated production of 5-hydroxymethylcytosine." *Nat Cell Biol.* 2015 May;17(5):545-57. doi: 10.1038/ncb3147. Epub 2015 Apr 27.
- Fortier, S., T. MacRae, et al. "Haploinsufficiency screen highlights two distinct groups of ribosomal protein genes essential for embryonic stem cell fate." *Proc Natl Acad Sci U S A.* 2015 Feb 17;112(7):2127-32. doi: 10.1073/pnas.1418845112. Epub 2015 Feb 2.
- Galoian, K., A. Qureshi, et al. "Epigenetic regulation of embryonic stem cell marker miR302C in human chondrosarcoma as determinant of antiproliferative activity of proline-rich polypeptide 1." *Int J Oncol.* 2015 Jun 18. doi: 10.3892/ijo.2015.3054.
- Ghanian, M. H., Z. Farzaneh, et al. "Nanotopographical control of human embryonic stem cell differentiation into definitive endoderm." *J Biomed Mater Res A.* 2015 Apr 22. doi: 10.1002/jbm.a.35483.
- Glass, C., R. Singla, et al. "Mouse embryonic stem cell-derived cardiac myocytes in a cell culture dish." *Methods Mol Biol.* 2015;1299:145-52. doi: 10.1007/978-1-4939-2572-8_11.
- Grealish, S., A. Heuer, et al. "Monosynaptic Tracing using Modified Rabies Virus Reveals Early and Extensive Circuit Integration of Human Embryonic Stem Cell-Derived Neurons." *Stem Cell Reports.* 2015 Jun 9;4(6):975-83. doi: 10.1016/j.stemcr.2015.04.011. Epub 2015 May 21.
- Hajizadeh-Saffar, E., Y. Tahamtani, et al. "Inducible VEGF expression by human embryonic stem cell-derived mesenchymal stromal cells reduces the minimal islet mass required to reverse diabetes." *Sci Rep.* 2015 Mar 30;5:9322. doi: 10.1038/srep09322.
- Hannes, T., M. Wolff, et al. "Electrophysiological characteristics of embryonic stem cell-derived cardiomyocytes are cell line-dependent." *Cell Physiol Biochem.* 2015;35(1):305-14. doi: 10.1159/000369697. Epub 2015 Jan 10.

16. Hoeber, J., C. Trolle, et al. "Human Embryonic Stem Cell-Derived Progenitors Assist Functional Sensory Axon Regeneration after Dorsal Root Avulsion Injury." *Sci Rep.* 2015 Jun 8;5:10666. doi: 10.1038/srep10666.
17. Hogan, M. S., D. E. Parfitt, et al. "Transient pairing of homologous Oct4 alleles accompanies the onset of embryonic stem cell differentiation." *Cell Stem Cell.* 2015 Mar 5;16(3):275-88. doi: 10.1016/j.stem.2015.02.001.
18. Holzspies, J., G. Dela Cruz, et al. "Resolving Heterogeneity: Fluorescence-Activated Cell Sorting of Dynamic Cell Populations from Feeder-Free Mouse Embryonic Stem Cell Culture." *Methods Mol Biol.* 2015 May 31.
19. Jacinto, F. V., C. Benner, et al. "The nucleoporin Nup153 regulates embryonic stem cell pluripotency through gene silencing." *Genes Dev.* 2015 Jun 15;29(12):1224-38. doi: 10.1101/gad.260919.115. Epub 2015 Jun 16.
20. Jacquet, L., A. Neueder, et al. "Three Huntington's Disease Specific Mutation-Carrying Human Embryonic Stem Cell Lines Have Stable Number of CAG Repeats upon In Vitro Differentiation into Cardiomyocytes." *PLoS One.* 2015 May 20;10(5):e0126860. doi: 10.1371/journal.pone.0126860. eCollection 2015.
21. Jenny, R. A., C. Hirst, et al. "Productive Infection of Human Embryonic Stem Cell-Derived NKX2.1+ Respiratory Progenitors With Human Rhinovirus." *Stem Cells Transl Med.* 2015 Jun;4(6):603-14. doi: 10.5966/sctm.2014-0274. Epub 2015 Apr 14.
22. Julienne, H., B. Audit, et al. "Embryonic stem cell specific "master" replication origins at the heart of the loss of pluripotency." *PLoS Comput Biol.* 2015 Feb 6;11(2):e1003969. doi: 10.1371/journal.pcbi.1003969. eCollection 2015 Feb.
23. Jyoti, S. and S. Tandon "Genetic basis for developmental toxicity due to statin intake using embryonic stem cell differentiation model." *Hum Exp Toxicol.* 2015 Feb 23. pii: 0960327114564795.
24. Khan, M., E. Nickoloff, et al. "Embryonic Stem Cell-Derived Exosomes Promote Endogenous Repair Mechanisms and Enhance Cardiac Function Following Myocardial Infarction." *Circ Res.* 2015 Jun 19;117(1):52-64. doi: 10.1161/CIRCRESAHA.117.305990. Epub 2015 Apr 22.
25. Kiris, E., J. E. Nuss, et al. "Phosphatase Inhibitors Function as Novel, Broad Spectrum Botulinum Neurotoxin Antagonists in Mouse and Human Embryonic Stem Cell-Derived Motor Neuron-Based Assays." *PLoS One.* 2015 Jun 10;10(6):e0129264. doi: 10.1371/journal.pone.0129264. eCollection 2015.
26. Ma H, Chen G. Stem cell. *The Journal of American Science* 2005;1(2):90-92.
27. Ma H, Cherng S. Eternal Life and Stem Cell. *Nature and Science.* 2007;5(1):81-96.
28. Ma H, Cherng S. Nature of Life. *Life Science Journal* 2005;2(1):7 - 15.
29. Ma H, Yang Y. Turrutopsis nutricula. *Nature and Science* 2010;8(2):15-20. http://www.sciencepub.net/nature/ns0802/03_1279_ho_ngbao_turrutopsis_ns0802_15_20.pdf.
30. Ma H. The Nature of Time and Space. *Nature and science* 2003;1(1):1-11. *Nature and science* 2007;5(1):81-96.
31. Matveeva, N. M., E. A. Kizilova, et al. "Generation of mouse chimeras with high contribution of tetraploid embryonic stem cells and embryonic stem cell-fibroblast hybrid cells." *Methods Mol Biol.* 2015;1313:61-71. doi: 10.1007/978-1-4939-2703-6_4.
32. Mehta, R. H. "Sourcing human embryos for embryonic stem cell lines: problems & perspectives." *Indian J Med Res.* 2014 Nov;140 Suppl:S106-11.
33. Melidoni, A. N., M. R. Dyson, et al. "Selection of Antibodies Interfering with Cell Surface Receptor Signaling Using Embryonic Stem Cell Differentiation." *Methods Mol Biol.* 2015 Jun 3.
34. Menasche, P., V. Vanneau, et al. "Human embryonic stem cell-derived cardiac progenitors for severe heart failure treatment: first clinical case report." *Eur Heart J.* 2015 May 19. pii: ehv189.
35. Michel-Schmidt, R., C. J. Kirkpatrick, et al. "Effect of LIF-withdrawal on acetylcholine synthesis in the embryonic stem cell line CGR8 is not mediated by STAT3, PI3Ks or cAMP/PKA pathways." *Int Immunopharmacol.* 2015 Apr 14. pii: S1567-5769(15)00160-5. doi: 10.1016/j.intimp.2015.04.005.
36. Morey, L., A. Santanach, et al. "Pluripotency and epigenetic factors in mouse embryonic stem cell fate regulation." *Mol Cell Biol.* 2015 Jun 1. pii: MCB.00266-15.
37. Movahednia, M. M., F. K. Kidwai, et al. "Differential effects of the extracellular microenvironment on human embryonic stem cell differentiation into keratinocytes and their subsequent replicative life span." *Tissue Eng Part A.* 2015 Apr;21(7-8):1432-43. doi: 10.1089/ten.TEA.2014.0551. Epub 2015 Mar 18.
38. Nakhaei-Rad, S., H. Nakhaeizadeh, et al. "The Function of Embryonic Stem Cell-expressed RAS (E-RAS), a Unique RAS Family Member, Correlates with Its Additional Motifs and Its Structural Properties." *J Biol Chem.* 2015 Jun 19;290(25):15892-903. doi: 10.1074/jbc.M115.640607. Epub 2015 May 4.
39. National Center for Biotechnology Information, U.S. National Library of Medicine. <http://www.ncbi.nlm.nih.gov/pubmed>. 2015.
40. Nichols, J. and T. Boroviak "Maximizing Clonal Embryonic Stem Cell Derivation by ERK Pathway Inhibition." *Methods Mol Biol.* 2015 May 31.
41. Orellana, M. D., G. C. De Santis, et al. "Efficient recovery of undifferentiated human embryonic stem cell cryopreserved with hydroxyethyl starch, dimethyl sulphoxide and serum replacement." *Cryobiology.* 2015 Jan 29. pii: S0011-2240(15)00023-1. doi: 10.1016/j.cryobiol.2015.01.005.
42. Ozeki, N., N. Hase, et al. "Interleukin-1beta-induced autophagy-related gene 5 regulates proliferation of embryonic stem cell-derived odontoblastic cells." *PLoS One.* 2015 Apr 20;10(4):e0124542. doi: 10.1371/journal.pone.0124542. eCollection 2015.
43. Pearson, S., S. Cuvertino, et al. "In vivo repopulating activity emerges at the onset of hematopoietic specification during embryonic stem cell differentiation." *Stem Cell Reports.* 2015 Mar

- 10:4(3):431-44. doi: 10.1016/j.stemcr.2015.01.003. Epub 2015 Feb 5.
44. Piao, J., T. Major, et al. "Human embryonic stem cell-derived oligodendrocyte progenitors remyelinate the brain and rescue behavioral deficits following radiation." *Cell Stem Cell*. 2015 Feb 5;16(2):198-210. doi: 10.1016/j.stem.2015.01.004.
 45. Piatti, P., C. Y. Lim, et al. "Embryonic stem cell differentiation requires full length Chd1." *Sci Rep*. 2015 Jan 26;5:8007. doi: 10.1038/srep08007.
 46. Ramos-Ibeas, P., E. Pericuesta, et al. "Characterisation of the deleted in azoospermia like (Dazl)-green fluorescent protein mouse model generated by a two-step embryonic stem cell-based strategy to identify pluripotent and germ cells." *Reprod Fertil Dev*. 2015 May 6. doi: 10.1071/RD14253.
 47. Robbez-Masson, L. and H. M. Rowe "Retrotransposons shape species-specific embryonic stem cell gene expression." *Retrovirology*. 2015 May 29;12:45. doi: 10.1186/s12977-015-0173-5.
 48. Sarkar, P., S. M. Randall, et al. "Activin/nodal signaling switches the terminal fate of human embryonic stem cell-derived trophoblasts." *J Biol Chem*. 2015 Apr 3;290(14):8834-48. doi: 10.1074/jbc.M114.620641. Epub 2015 Feb 10.
 49. Schmitt, J., S. Eckardt, et al. "Human parthenogenetic embryonic stem cell-derived neural stem cells express HLA-G and show unique resistance to NK cell-mediated killing." *Mol Med*. 2015 Mar 23. doi: 10.2119/molmed.2014.00188.
 50. Schulpen, S. H., J. L. Pennings, et al. "Gene Expression Regulation and Pathway Analysis After Valproic Acid and Carbamazepine Exposure in a Human Embryonic Stem Cell-Based Neurodevelopmental Toxicity Assay." *Toxicol Sci*. 2015 May 15. pii: kfv094.
 51. Sikorski, D. J., N. J. Caron, et al. "Clonal analysis of individual human embryonic stem cell differentiation patterns in microfluidic cultures." *Biotechnol J*. 2015 Jun 9. doi: 10.1002/biot.201500035.
 52. Siriboon, C., Y. H. Lin, et al. "Putative porcine embryonic stem cell lines derived from aggregated four-celled cloned embryos produced by oocyte bisection cloning." *PLoS One*. 2015 Feb 13;10(2):e0118165. doi: 10.1371/journal.pone.0118165. eCollection 2015.
 53. Sivaraman, M. A. and S. N. Noor "Human Embryonic Stem Cell Research: Ethical Views of Buddhist, Hindu and Catholic Leaders in Malaysia." *Sci Eng Ethics*. 2015 Jun 7.
 54. Skarnes, W. C. "Is mouse embryonic stem cell technology obsolete?" *Genome Biol*. 2015 May 27;16:109. doi: 10.1186/s13059-015-0673-6.
 55. Song, W. K., K. M. Park, et al. "Treatment of macular degeneration using embryonic stem cell-derived retinal pigment epithelium: preliminary results in asian patients." *Stem Cell Reports*. 2015 May 12;4(5):860-72. doi: 10.1016/j.stemcr.2015.04.005. Epub 2015 Apr 30.
 56. Sorkio, A., P. J. Porter, et al. "Surface modified biodegradable electrospun membranes as a carrier for human embryonic stem cell derived retinal pigment epithelial cells." *Tissue Eng Part A*. 2015 May 6.
 57. Tosca, L., O. Feraud, et al. "Genomic instability of human embryonic stem cell lines using different passaging culture methods." *Mol Cytogenet*. 2015 Apr 23;8:30. doi: 10.1186/s13039-015-0133-8. eCollection 2015.
 58. Vairo, L., E. Medei, et al. "Functional properties of a Brazilian derived mouse embryonic stem cell line." *An Acad Bras Cienc*. 2015 Mar;87(1):275-88. doi: 10.1590/0001-3765201520140474. Epub 2015 Mar 6.
 59. Vakili, K., A. M. McGahan, et al. "Progress in human embryonic stem cell research in the United States between 2001 and 2010." *PLoS One*. 2015 Mar 26;10(3):e0120052. doi: 10.1371/journal.pone.0120052. eCollection 2015.
 60. Van der Jeught, M., J. Taelman, et al. "Application Of Small Molecules Favoring Naive Pluripotency during Human Embryonic Stem Cell Derivation." *Cell Reprogram*. 2015 Jun;17(3):170-80. doi: 10.1089/cell.2014.0085.
 61. Weber, M., D. Schlembach, et al. "PP044. Differential expression of trophoblast-, endothelial- and embryonic stem cell-associated transcription factors in 1st trimester, and in 3rd trimester preeclampsia and intrauterine growth restriction." *Pregnancy Hypertens*. 2013 Apr;3(2):82. doi: 10.1016/j.preghy.2013.04.071. Epub 2013 Jun 6.
 62. Werneburg, S., F. F. Buettner, et al. "Polysialic acid modification of the synaptic cell adhesion molecule SynCAM 1 in human embryonic stem cell-derived oligodendrocyte precursor cells." *Stem Cell Res*. 2015 May;14(3):339-46. doi: 10.1016/j.scr.2015.03.001. Epub 2015 Mar 21.
 63. Wettstein, R., M. Bodak, et al. "Generation of a Knockout Mouse Embryonic Stem Cell Line Using a Paired CRISPR/Cas9 Genome Engineering Tool." *Methods Mol Biol*. 2015 Mar 12.
 64. Wikipedia. The free encyclopedia. <http://en.wikipedia.org>. 2015.
 65. Zarei Fard, N., T. Talaei-Khozani, et al. "Comparison of cell viability and embryoid body size of two embryonic stem cell lines after different exposure times to bone morphogenetic protein 4." *Iran J Med Sci*. 2015 Mar;40(2):110-7.