TELOMERES AND TELOMERASE IN ADULT STEM CELLS AND PLURIPOTENT EMBRYONIC STEM pdf

1: Stem Cells & Telomeres by Sarah Jamal-Eddine on Prezi

Telomeres and telomerase in adult stem cells and pluripotent embryonic stem cells. MariÃ³n RM(1), Blasco MA. Author information: (1)Telomeres and Telomerase Group, Molecular Oncology Program, Spanish National Cancer Centre (CNIO), Melchor FernÃ¡ndez Almagro 3, Madrid, E, Spain.

The enzyme consists of a protein component with reverse transcriptase activity, encoded by this gene, and an RNA component that serves as a template for the telomere repeat. Telomerase expression plays a role in cellular senescence, as it is normally repressed in postnatal somatic cells , resulting in progressive shortening of telomeres. Studies in mice suggest that telomerase also participates in chromosomal repair, since de novo synthesis of telomere repeats may occur at double-stranded breaks. Alternatively spliced variants encoding different isoforms of telomerase reverse transcriptase have been identified; the full-length sequence of some variants has not been determined. Alternative splicing at this locus is thought to be one mechanism of regulation of telomerase activity. Meanwhile, over-expression of hTERT is often associated with cancers and tumor formation. Stem cells[edit] hTERT in stem cells[edit] hTERT is often up-regulated in cells that divide rapidly, including both embryonic stem cells and adult stem cells. Therefore, it is responsible for the self-renewal properties of stem cells. Telomerase are found specifically to target shorter telomere over longer telomere, due to various regulatory mechanisms inside the cells that reduce the affinity of telomerase to longer telomeres. This preferential affinity maintains a balance within the cell such that the telomeres are of sufficient length for their function and yet, at the same time, not contribute to aberrant telomere elongation [20] High expression of hTERT is also often used as a landmark for pluripotency and multipotency state of embryonic and adult stem cells. Over-expression of hTERT was found to immortalize certain cell types as well as impart different interesting properties to different stem cells. Hence, hTERT acts as the limiting factor for telomerase activity in differentiated cells [14] [23] However, with hTERT over-expression, active telomerase can be formed in differentiated cells. This method has been used to immortalize prostate epithelial and stromal-derived cells, which are typically difficult to culture in vitro. The expression profile of mesenchymal stem cells converges towards embryonic stem cells, suggesting that these cells may have embryonic stem cell-like properties. However, it has been observed that mesenchymal stem cells undergo decreased levels of spontaneous differentiation. Therefore, over-expression of hTERT, which is akin to increasing telomerase activities, may create adult stem cells with a larger capacity for differentiation and hence, a larger capacity for treatment. Increasing the telomerase activities in stem cells gives different effects depending on the intrinsic nature of the different types of stem cells. The survival of these stem cells was enhanced, although there was no increase in the amount of population doubling. The enzyme complex acts through the addition of telomeric repeats to the ends of chromosomal DNA. This generates immortal cancer cells. Normal somatic cells , on the other hand, do not have detectable telomerase activity. The hTERT gene has been examined for mutations and their association with the risk of contracting cancer. Over two hundred combinations of hTERT polymorphisms and cancer development have been found. Glycogen synthase kinase 3 GSK3 seems to be over-expressed in most cancer cells. Since normal somatic cells do not express TERT, telomerase inhibition in cancer cells can cause senescence and apoptosis without affecting normal human cells. Telomere length in healthy adult cells elongates and acquires epigenetic characteristics similar to those of ES cells when reprogrammed as iPS cells. Some epigenetic characteristics of ES cells include a low density of tri-methylated histones H3K9 and H4K20 at telomeres, as well as an increased detectable amount of TERT transcripts and protein activity. Cells restricted to a specific lineage are capable of division only a set number of times, set by the length of telomeres, before they senesce. The protein can be a toxin, an apoptotic factor, or a viral protein. Toxins such as diphtheria toxin interfere with cellular processes and eventually induce apoptosis.

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2: Scientists find that for stem cells to be healthy, telomere length has to be just right

Telomerase expression is silenced in most adult somatic tissues with the exception of adult stem cell (SC) compartments, which have the property of having the longest telomeres within a given tissue.

Here are some stem cell stories that caught our eye this past week. Some are groundbreaking science, others are of personal interest to us, and still others are just fun. Telomeres and stem cell stability: Because of the way DNA replication works, the telomeres shorten each time a cell divides. Trim away enough of the telomere over time and, like a frayed shoelace, the chromosomes become unstable and an easy target for damage which eventually leads to cell death. Telomeres white dots form a protective cap on chromsomes gray. Wikimedia Stem cells are unique in that they contain an enzyme called telomerase that lengthens telomeres. But Salk Institute scientists reported this week that too much telomere can be just as bad, if not worse, than too little. Cells with low telomerase activity had shorter telomeres and died. Based on those results, the team was expecting cells with boosted telomerase activity and, in turn, extended telomeres would be especially stable. These experiments question the generally accepted notion that artificially increasing telomeres could lengthen life or improve the health of an organism. First author Teresa Rivera pointed out the big picture significance of this finding: Understanding how telomere length is regulated is an important step toward realizing the promise of stem cell therapies and regenerative medicine. That ground-breaking discovery ten years ago has opened the way for researchers worldwide to specialize iPS cells into all sorts of cell types from nerve cells to liver cells. While some cell types are easy to generate this way, others are much more difficult. Reporting this week in PNAS, a University of Wisconsinâ€"Madison research team has developed a nifty systematic, high-throughput method for identifying the factors necessary to convert a cell from one type to another. Their strategy promises to free researchers from the costly and time consuming trial and error approach still in use today. The centerpiece of their method is artificial transcription factors ATFs. Their impact on gene activity, in turn, can have a cascading effects on other genes and proteins ultimately causing, say a stem cell, to start making muscle proteins and turn into a muscle cell. Transcription factors are very modular proteins â€" one part is responsible for binding DNA, another part for affecting gene activity and other parts that bind to other proteins. The ATFs generated in this study are like lego versions of natural transcription factors â€" each are constructed from combinations of different transcription factor parts. The team made nearly 3 million different ATFs. They inserted the ATFs into skin cells that already had 3 of the 4 Yamanaka factors, they left out Oct4. They successfully generated iPS with this approach and then went back and studied the makeup of the ATFs that had caused cells to reprogram into iPS cells. Senior author Aseem Ansari gave a great analogy in a university press release: We test all those keys in parallel and when we see the motor fire up, we go back to see exactly which key switched it on. This finding is important because it suggests that future cell conversion experiments could uncover some not so obvious cell fate pathways. Ansari explains this point further: Those differences may lead to potential paths to developing a therapy. IPF is a chronic lung disease which causes scarring, or fibrosis, in the air sacs of the lung. This is the spot where oxygen is taken up by tiny blood vessels that surround the air sacs. With fibrosis, the air sacs stiffen and thicken and as a result less oxygen gets diffused into the blood and starves the body of oxygen. IPF can lead to death within 2 to 5 years after diagnosis. Unfortunately, no cures exist and the cause is unknown, or idiopathic. Wikimedia The transfer of oxygen from air sacs to blood vessels is an intricate one with many cell types involved. So pinpointing what goes wrong in IPF at a cellular and molecular level has proved difficult. In the current study, the scientists, for the first time, collected gene sequencing data from single cells from healthy and diseased lungs. This way, a precise cell by cell analysis of gene activity was possible. One set of gene activity patterns found in healthy sample were connected to proper formation of a particular type of air sac cell called the aveolar type 2 lung cell. Other gene patterns were linked to abnormal IPF cell types. With this data in hand, the researchers can further investigate the role of these genes in IPF which may open up new therapy approaches to this deadly

disease.

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3: telomeres | The Stem Cellar

Thus, telomerase activity and telomere maintenance are associated with the immortality of cancer cells, germ-line cells, and embryonic stem (ES) cells. In most human somatic cells except for stem cells and lymphocytes, telomerase activity is diminished after birth so that telomere length shortens with each cell division.

Reprogramming A scheme of the generation of induced pluripotent stem IPS cells. Red cells indicate the cells expressing the exogenous genes. The original set of reprogramming factors also dubbed Yamanaka factors are the transcription factors Oct4 Pou5f1 , Sox2 , cMyc , and Klf4. While this combination is most conventional in producing iPSCs, each of the factors can be functionally replaced by related transcription factors, miRNAs , small molecules, or even non-related genes such as lineage specifiers. However, considerable advances have been made in improving the efficiency and the time it takes to obtain iPSCs. Upon introduction of reprogramming factors, cells begin to form colonies that resemble pluripotent stem cells, which can be isolated based on their morphology, conditions that select for their growth, or through expression of surface markers or reporter genes. They chose twenty-four genes previously identified as important in ESCs and used retroviruses to deliver these genes to mouse fibroblasts. The fibroblasts were engineered so that any cells reactivating the ESC-specific gene, Fbx15 , could be isolated using antibiotic selection. Upon delivery of all twenty-four factors, ESC-like colonies emerged that reactivated the Fbx15 reporter and could propagate indefinitely. To identify the genes necessary for reprogramming, the researchers removed one factor at a time from the pool of twenty-four. By this process, they identified four factors, Oct4, Sox2, cMyc, and Klf4, which were each necessary and together sufficient to generate ESC-like colonies under selection for reactivation of Fbx These second-generation iPSCs were derived from mouse fibroblasts by retroviral-mediated expression of the same four transcription factors Oct4, Sox2, cMyc, Klf4. However, instead of using Fbx15 to select for pluripotent cells, the researchers used Nanog , a gene that is functionally important in ESCs. Additional genes, however, including certain members of the Klf family Klf1, Klf2, Klf4, and Klf5 , the Myc family c-myc, L-myc, and N-myc , Nanog , and LIN28 , have been identified to increase the induction efficiency. While Sox2 was the initial gene used for induction by Yamanaka et al. Sox1 yields iPS cells with a similar efficiency as Sox2, and genes Sox3 , Sox15 , and Sox18 also generate iPS cells, although with decreased efficiency. Klf4 of the Klf family of transcription factors was initially identified by Yamanaka et al. However, Thomson et al. Klf2 and Klf4 were found to be factors capable of generating iPS cells, and related genes Klf1 and Klf5 did as well, although with reduced efficiency. The Myc family of transcription factors are proto-oncogenes implicated in cancer. N-myc and L-myc have been identified to induce instead of c-myc with similar efficiency. Therefore, it was surprising when Yamanaka et al. LIN28 is an mRNA binding protein [21] expressed in embryonic stem cells and embryonic carcinoma cells associated with differentiation and proliferation. It poses numerous advantages when used instead of C-myc. However, recently a path was found for efficient reprogramming which required downregulation of the nucleosome remodeling and deacetylation NuRD complex. Plasmids , adenoviruses , and transposon vectors have all been explored, but these often come with the tradeoff of lower throughput. Depending on the methods used, reprogramming of adult cells to obtain iPSCs may pose significant risks that could limit their use in humans. For example, if viruses are used to genomically alter the cells, the expression of oncogenes cancer-causing genes may potentially be triggered. In February , scientists announced the discovery of a technique that could remove oncogenes after the induction of pluripotency, thereby increasing the potential use of iPS cells in human diseases. This is particularly challenging because the genome-wide epigenetic code must be reformatted to that of the target cell type in order to fully reprogram a cell. However, three separate groups were able to find mouse embryonic fibroblast MEF -derived iPS cells that could be injected into tetraploid blastocysts and resulted in the live birth of mice derived entirely from iPS cells, thus ending the debate over the equivalence of embryonic stem cells ESCs and iPS with regard to pluripotency. Rows of similar colors represent studies that used similar strategies for

reprogramming. This timeline summarizes the key strategies and techniques used to develop iPS cells in the first five years after Yamanaka et al. Alternative approaches[edit] Mimicking transcription factors with chemicals[edit] One of the main strategies for avoiding problems 1 and 2 has been to use minute compounds that can mimic the effects of transcription factors. These molecule compounds can compensate for a reprogramming factor that does not effectively target the genome or fails at reprogramming for another reason; thus they raise reprogramming efficiency. They also avoid the problem of genomic integration, which in some cases contributes to tumor genesis. Key studies using such strategy were conducted in A similar type of compensation mechanism was proposed to mimic the effects of Sox2. In , Ding et al. They used a cocktail of seven small-molecule compounds including DZNep to induce the mouse somatic cells into stem cells which they called CiPS cells with the efficiency â€" at 0. The CiPS cells were introduced into developing mouse embryos and were found to contribute to all major cells types, proving its pluripotency. Adding a third compound known to be involved in the cell survival pathway, Thiazovivin further increases the efficiency by fold. Using the combination of these three compounds also decreased the reprogramming process of the human fibroblasts from four weeks to two weeks. Alternate vectors[edit] Another key strategy for avoiding problems such as tumor genesis and low throughput has been to use alternate forms of vectors: In , Hochedlinger et al. The adenovirus is unique from other vectors like viruses and retroviruses because it does not incorporate any of its own genes into the targeted host and avoids the potential for insertional mutagenesis. Also in , Yamanaka et al. Although the plasmid methods avoid viruses, they still require cancer-promoting genes to accomplish reprogramming. The other main issue with these methods is that they tend to be much less efficient compared to retroviral methods. Furthermore, transfected plasmids have been shown to integrate into the host genome and therefore they still pose the risk of insertional mutagenesis. Because non-retroviral approaches have demonstrated such low efficiency levels, researchers have attempted to effectively rescue the technique with what is known as the PiggyBac Transposon System. Several studies have demonstrated that this system can effectively deliver the key reprogramming factors without leaving footprint mutations in the host cell genome. The PiggyBac Transposon System involves the re-excision of exogenous genes, which eliminates the issue of insertional mutagenesis. Stimulus-triggered acquisition of pluripotency In January , two articles were published claiming that a type of pluripotent stem cell can be generated by subjecting the cells to certain types of stress bacterial toxin, a low pH of 5. Measuring variations in microRNA expression in iPS cells can be used to predict their differentiation potential. Several mechanisms have been proposed. Induced pluripotent stem cells are similar to natural pluripotent stem cells, such as embryonic stem ES cells , in many aspects, such as the expression of certain stem cell genes and proteins, chromatin methylation patterns, doubling time, embryoid body formation, teratoma formation, viable chimera formation, and potency and differentiability, but the full extent of their relation to natural pluripotent stem cells is still being assessed. Cellular biological properties Morphology: Each cell had round shape, large nucleolus and scant cytoplasm. Doubling time and mitotic activity are cornerstones of ESCs, as stem cells must self-renew as part of their definition. The presence of catecholamine -associated enzymes may indicate that iPSCs, like hESCs, may be differentiable into dopaminergic neurons. Stem cell-associated genes were downregulated after differentiation. Teratomas are tumors of multiple lineages containing tissue derived from the three germ layers endoderm , mesoderm and ectoderm ; this is unlike other tumors, which typically are of only one cell type. Teratoma formation is a landmark test for pluripotency. The hollow trophoblast is unable to form a living embryo, and thus it is necessary for the embryonic stem cells within the embryoblast to differentiate and form the embryo. Chimeric living mouse pups were created: Widespread methylation of a gene interferes with expression by preventing the activity of expression proteins, or by recruiting enzymes that interfere with expression. Thus, methylation of a gene effectively silences it by preventing transcription. Human iPS cells are highly similar to ES cells in their patterns of which cytosines are methylated , more than to any other cell type. However, on the order of a thousand sites show differences in several iPS cell lines. Half of these resemble the somatic cell line the iPS cells were derived from, the rest are iPSC-specific. Tens of regions which are megabases in size have

also been found where iPS cells are not reprogrammed to the ES cell state. Histones are compacting proteins that are structurally localized to DNA sequences that can affect their activity through various chromatin-related modifications. Safety[edit] The major concern with the potential clinical application of iPSCs is their propensity to form tumors. Teratoma formation is considered a major obstacle to stem-cell based regenerative medicine by the FDA. A more recent study on motor functional recovery after spinal cord injuries in mice showed that after human-induced pluripotent stem cells were transplanted into the mice, the cells differentiated into three neural lineages in the spinal cord. The cells stimulated regrowth of the damaged spinal cord, maintained myelination, and formed synapses. These positive outcomes were observed for over days after the spinal cord injury, without tumor formation. All the genes that have been shown to promote iPSC formation have also been linked to cancer in one way or another. Some of the genes are known oncogenes, including the members of the Myc family. A non-genetic method of producing iPSCs has been demonstrated using recombinant proteins, but its efficiency was quite low. Other approaches such as using adenovirus or plasmids are generally thought to be safer than retroviral methods. An important area for future studies in the iPSC field is directly testing iPSC tumorigenicity using methods that mimic the approaches that would be used for regenerative medicine therapies. Such studies are crucial since iPSCs not only form teratoma, but also mice derived from iPSCs have a high incidence of death from malignant cancer. When a similar procedure was performed on genetically equivalent ES cells however, Zhou et al. They took cells from a chimera that had been grown from IPSC clones and a mouse embryo, this tissue was then transplanted into syngenic mice. Findings indicate that there was no significant difference in the immunogenic response produced by the IPS cells and the ES cells. Furthermore, Araki et al. Recent achievements and future tasks for safe iPSC-based cell therapy are collected in the review of Okano et al. A key tradeoff to overcome is that between efficiency and genomic integration. Most methods that do not rely on the integration of transgenes are inefficient, while those that do rely on the integration of transgenes face the problems of incomplete reprogramming and tumor genesis, although a vast number of techniques and methods have been attempted. Another large set of strategies is to perform a proteomic characterization of iPS cells. One approach might attempt to combine the positive attributes of these strategies into an ultimately effective technique for reprogramming cells to iPS cells. Another approach is the use of iPS cells derived from patients to identify therapeutic drugs able to rescue a phenotype. For instance, iPS cell lines derived from patients affected by ectodermal dysplasia syndrome EEC , in which the p63 gene is mutated, display abnormal epithelial commitment that could be partially rescued by a small compound [65] Disease modelling and drug development[edit] An attractive feature of human iPS cells is the ability to derive them from adult patients to study the cellular basis of human disease. Since iPS cells are self-renewing and pluripotent, they represent a theoretically unlimited source of patient-derived cells which can be turned into any type of cell in the body. This is particularly important because many other types of human cells derived from patients tend to stop growing after a few passages in laboratory culture. Managed by the University of Oxford , the effort pooled funds and resources from 10 pharmaceutical companies and 23 universities. The goal is to generate a library of 1, iPS cell lines which will be used in early drug testing by providing a simulated human disease environment.

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4: Telomere and telomerase in stem cells

This parallels the decreased stem cell functionality of different adult stem cell compartments of telomerase-deficient mice, including neural stem cells, epidermal stem cells, and hematopoietic stem cells.

This is an open access article distributed under the Creative Commons Attribution License , which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. Short telomeres have been associated with many age-related conditions in humans, and genetic mutations resulting in short telomeres in humans manifest as syndromes of precocious aging. Thus, telomere length must be reset with each subsequent generation. Although telomerase is purportedly responsible for restoring telomere DNA, recent studies have elucidated the role of alternative telomeres lengthening mechanisms in the reprogramming of early embryos and stem cells, which we review here. Telomeres prevent chromosome end-joining and ward off DNA damage repair machinery that would otherwise inappropriately repair the telomere as a double-stranded DNA break. In humans, most tissues exhibit a marked decrease in telomere reserve over time [1 , 2]. Cell division, oxidative damage, and genotoxic insults can directly or by way of DNA damage repair responses reduce the amount of DNA capping the chromosome end. Excessive telomere shortening induces cell senescence and eventually apoptosis [3]. Telomere DNA can be replenished by telomerase [3 â€” 5], a reverse-transcriptase-acting holoenzyme that adds a modest 50â€” bp per round of cell division. Expression of telomerase in humans is limited to highly proliferative tissues which harbor progenitor or stem cell compartments. In contrast to humans, many model organisms such as outbred mouse strains have constitutive telomerase expression coincident with longer telomeres. Recombination-mediated alternative lengthening of telomeres ALT pathway s have been proposed [6 â€” 8], which can augment telomere length by thousands of base-pairs within a few cell cycles [9]. Telomere biology is an emerging field that holds great promise for advancing clinical medicine, particularly for aging and age-related diseases. Short telomeres have been associated with the gamut of age-related diseases, including diabetes mellitus [10 , 11], cardiovascular disease [12], liver disorders [13 , 14], cancer [15 â€” 19], and death from all causes [20]. Moreover, long telomeres have been associated with exceptional longevity and increased lifespan [21]. Chemical or genetic depletion of telomere length recapitulates many of these pathologies in mouse models. Direct evidence for the importance of telomere length in human disease derives from patients with mutations in TERT and TERC, the genes encoding the reverse transcriptase and RNA component of telomerase, respectively. This disorder manifests in tissues prone to high turnover, such as liver, fingernail beds, mucous membranes, and the hematopoietic system [16]. Symptoms arise when telomere lengths become critically short, which limits the ability of progenitor cells to maintain the differentiated cell populations in these tissues, leading to eventual organ system failure. Further, patients have a higher risk of developing cancer [28], particularly in those organs susceptible to telomere attrition. In families with inherited germline mutations in TERC and TERT, telomere length across generations is inversely related to the severity of disease manifestations. This genetic anticipation has also been documented in hereditary cancers [15]. Cellular reprogramming, resetting the aging process, requires purging of aged maternal proteins, degradation of maternal transcripts, resetting methylation cues, and rejuvenation of the genome and other cellular components. One paradigm to model this is somatic cell nuclear transfer SCNT , where an adult cell is transplanted into an enucleated oocyte. Interestingly, though Dolly the Sheep, the first mammal cloned by SCNT, exhibited signs of precocious aging, presumably due to incompletely reset telomeres inherited from the donor somatic cell. Here, we will review in vivo and in vitro data on telomere length reprogramming, with particular emphasis on systems with the greatest promise for the future of personalized medicine. Telomere Lengthening in Embryos Mammalian oocytes arrest following the extrusion of the first polar body and prior to completion of meiosis II. Fertilization by a competent sperm leads to the extrusion of the second polar body, rendering the oocyte haploid to complement the incoming paternal genome. What is known about telomere

dynamics during this period is largely limited to observational studies, both in mouse and human. High reactive oxygen species, generalized aging, and lack of telomerase activity conspire to reduce telomere length in oocytes [30 , 32 , 33]. In contrast, sperm are of the few cell types documented to elongate telomeres over the human lifespan [34 â€” 37], presumably due to the effects of telomerase activity in spermatogonia throughout the life of the male. Following fertilization and activation of the egg, embryonic cells undergo dramatic telomere lengthening [9 , 31 , 38 , 39]. In mouse, telomere length increases by thousands of base-pairs within the first one or two cell cycles [9], nearly doubling from the baseline oocyte length to the two-cell stage. Notably, telomerase activity remains undetectable in these cells. Instead, embryos exhibit high rates of telomere sister chromatid exchange T-SCE , telomere-specific localization of recombination proteins, and a favorable chromatin state. This effect remains robust in telomerase knock-out mice, suggesting an ALT-dependent mechanism at play in preimplantation mammalian development. Moreover, the lengthening takes place in parthenogenetically activated eggs, which lack sperm input during activation, suggesting that the capacity for telomere length reprogramming resides in the oocyte. Whereas telomerase-independent, recombination-based telomere elongation takes place during early preimplantation development, telomerase activity increases dramatically at the blastocyst stage of development see Figure 1. The greatest degree of telomere lengthening occurs in the inner cell mass, the pluripotent cells giving rise to the embryo proper [40], in contrast to the trophectoderm, which gives rise to tissues that will become placenta and other extra embryonic tissues. Interestingly, telomere length in the inner cell mass is substantially shorter than that of embryonic stem cells, which lengthen after several passages in culture. Studies of telomere length dynamics during early embryo development and blastocyst development and during formation of ESCs should be conducted on human tissue. Schematic of telomere length reprogramming in mammalian embryonic development. The greatest telomere lengthening takes place during the earliest stages of preimplantation development, the cleavage-stage embryo, which may coincide with zygote genome activation. Later, in development and adult life, telomerase becomes the dominant telomere maintenance mechanism for the inner cell mass and in tissue-specific telomere replenishment in stem cell niches. Owing to the limitations in the United States for using human embryos in research, these studies have not been directly repeated in humans. However, one group based in the United Kingdom attempted to resolve telomere length at the level of the individual chromosome by utilizing semiquantitative fluorescent in situ hybridization QFISH with individual gametes and pronuclear embryos [41 , 42]. This group found that telomere length is shorter in sperm than oocytes, including in immature oocytes and mature oocytes and at the pronuclear stage. The results of these experiments are open to interpretation for several reasons. Until fertilization, immature and mature oocytes contain twice the amount of DNA as sperm. Moreover, QFISH usually requires metaphase spreads to measure telomere length, so the use of individual interphase spreads needs to be validated. Sperm and oocyte telomere length discrepancies found by this method could also be accounted for by differing chromatin states that allow the probe to have more ready access to the oocyte pronucleus over the highly condensed male pronucleus. Assisted reproductive technologies afford the opportunity to directly access human gametes and embryos, allowing for such studies of telomere length dynamics during human preimplantation development. Such studies have revealed shorter telomeres in oocytes of women who do not conceive following IVF compared to those who do [29] and in oocytes from cycles producing fragmented embryos [43]. Moreover, shorter telomere length was found in aneuploid blastomeres and polar bodies than in euploid cells from the same IVF patient and cycle [39], which is consistent with similar findings in mouse models with short telomeres [44]. Indeed, a wide range of complications of advanced reproductive age have been associated with shorter telomere length, including Down syndrome [45] and recurrent miscarriage [46] also reviewed in [30]. Although the studies provide a unique insight into events during telomere reprogramming, these studies by necessity remain observational. Interestingly, a variety of studies have revealed basic reprogramming capacity in offspring from somatic cell nuclear transfer SCNT , which creates a cloned embryo, such as Dolly the Sheep. Since the telomeres in somatic cells in most mammalian species exhibit age-related decline, use of

SCNT-cloned animals provides an interesting sample of reprogramming across species. Newborn clones of pig and cattle exhibit normal telomere lengthening during embryonic reprogramming, although the rate of production of viable, full-term offspring by way of SCNT is very low [47 â€" 51]. Minor discrepancies in the degree of telomere lengthening amongst these studies might be explained by the small sample sizes and the differential time points of tissue selection for telomere length analysis. Overall, these studies are consistent with observational studies in human and experimental studies in mouse that demonstrated a minimum telomere length is likely required for the development of a competent embryo; to determine this experimentally, it would be intriguing to increase telomere length and examine efficiency of SCNT and production of cloned embryos. Telomere Length Reprogramming in Stem Cells Stem cells are defined not only by their differentiation potential but also by their capacity for unlimited self-replication. The need for prolonged self-replication requires adequate telomere length and telomere maintenance, which can limit the powerful new methods available for generating induced pluripotent cells. IPSCs lacking sufficient telomere length fail to achieve germline transmission or tetraploid complementation, the most stringent tests of pluripotency, and cannot be maintained in culture over long periods. This might have contributed, in part, to the variable quality of iPSCs during early efforts by the Yamanaka group and the initial failure of these cells to contribute to chimeras and may ultimately limit the future application of iPSCs in regenerative medicine [52 , 53]. To correct this, present efforts in the field of iPSCs have strived to improve the quality of iPSC generated by focusing on telomere dynamics during the process of reprogramming. Since expression of the common pluripotency markers Oct4, Nanog, Sox2, Lin28, etc. Identifying lack of true pluripotency in iPSC with short telomeres recapitulated earlier suggestions linking telomere length with detection of pluripotent status. This study also revealed an increased rate of telomere sister chromatid exchange T-SCE in the absence of full telomerase function, demonstrating a telomerase-independent mechanism ALT at work during reprogramming. In particular, this study implicated limited telomerase function possibly arising from poor activation by pluripotency factors and reactivation of exogenous pluripotency factors in response to loss of full telomerase function, in addition to ALT in contributing to telomere length variability in iPSC reprogramming. Recombination events such as T-SCE require reductions in DNA methylation and epigenetic remodeling and are frequently associated with genomic stability [56]. Understanding the epigenetic mechanisms regulating telomere length is critical for improving the quality of iPSC. Rescue of stable differentiation was achieved by restoration of Dnmt3b or inhibition of Nanog resulting inreversed global DNA hypomethylation and normal repression of Nanog by de novo methylation. This work led to a model in which adequate telomere length directly impacts the ability of cells to maintain stable differentiation capacity. Zscan4 is emerging as a potentially important factor in generating high-quality iPSCs. First identified for its essential role in development from 2-cell to 4-cell embryos [58], ZScan4 was later shown to play a role in ESC regulation. Importantly, a Zscan4-positive state has been observed in ESC populations, where it colocalizes to telomeres and accompanies telomere elongation [59]. Moreover, ZScan4 knockdown accompanied reduced proliferation, decreased T-SCE, decreased telomere length, and abnormal karyotype. Over subsequent passages, the majority of Zscan4-deficient cells became cell cycle arrested. Taken together, these findings point to an essential role for Zscan4 in regulation of telomere length in order to perhaps protect cells from genomic instability. More recent work has elucidated the regulatory relationship between Tbx3 and Zscan4, namely, that Tbx3 can activate Zscan4 by inhibiting DNA methylation at subtelomeric regions, leading to depression of genes in this region e. The addition of Zscan4 or Tbx3 to the reprogramming protocol produces iPSC able to pass the most stringent tests of pluripotency and pushes closer toward the development of a protocol for iPSC suitable for clinical application [61]. In humans, the nature of telomere reprogramming has recapitulated many findings in other model organisms. Generation of human iPSC hiPSC from patient cells with the telomere disorder Dyskeratosis Congenita demonstrated the ability to elongate telomeres after reprogramming [62]. While in early passages these dyskerin-mutated DKC1 hiPSC lines showed gradual telomere reduction, prolonged culture enabled telomere resetting to match the length of parent fibroblasts,

indicating that the TERC deficiency within these cells had been overcome by the reprograming process. Other work with DKC1 hiPSC revealed that extended culture led to telomere shortening and spontaneous differentiation [63]. Additionally, hiPSC from patients with mutations in telomerase itself TCAB1 that cause mislocalization of the enzyme fails to lengthen telomeres despite having normal telomerase activity, indicating that proper localization is as critical a factor as activity for telomere maintenance. Combined, these data reinforce that many factors affecting telomere length and telomere maintenance ultimately impact pluripotency and self-renewal of human and animal cells. SCNT would provide the gold standard for production of personalized stem cells for humans, as the resulting progeny are veritable clones of the donor genome. Although methods for deriving SCNT stem cells were conceived over a decade ago, this was only successfully completed in humans very recently due to excessive technical difficulty and ethical limitations [64]. The data suggest that SCNT produces superior pluripotent cells and that the process can rescue differentiation ability in cells with critically short telomeres. Still, the requirement for high-quality human oocytes for SCNT will likely drive more in-depth studies into the factors within the oocyte necessary to produce superior stem cells [65]. These factors, once isolated, will likely have an explosive impact on Yamanaka reprogramming both in terms of the quality of the cells and the efficiency of the process. Discussion Advances in modern medicine have led to remarkable prolongation of human life span. Concomitantly, an increase in delayed child rearing until later in life has led to reduced fecundity. As a result, the burden of age-related diseases and infertility makes elucidation of the pathobiology of aging an urgent priority. IPSCs bypass the ethnical and immune-compatibility issues that limit application of embryonic stem cells for therapeutic purposes. Not only do these iPSCs promise to provide advanced models and treatments of complex human diseases, but the efficiency of reprogramming, especially pertaining to telomeres, must be fully addressed before clinical applications can be considered. In human reproduction, a minimum telomere length is likely required for production of a competent embryo, as in the development of pluripotent cell lines.

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5: Stem Cell Passaging Yields Mice with Hyper-Long Telomeres

This chapter will focus on the current knowledge of the role of telomeres and telomerase in adult SC, as well as during nuclear reprograming to generate pluripotent embryonic-like stem cells from adult differentiated cells.

What do science and improv have in common? The answer is not a whole lot. I met Jill at the Bridges Conference in July and was immediately impressed by her passion for science and communications. I was also intrigued by her interest in improv and how she balances her time between two very different passions. What did you study during your Bridges internship? I was a research intern in the lab of Dr. In his lab, we study telomeres, which are the pieces of DNA at the end of chromosomes that help protect them from being degraded. We do big protein pull downs to try to figure out what new and novel proteins are surrounding the mechanisms that maintain telomere function, and then we do functional assays to figure out what these proteins do. One function of telomeres is that they suppress the double and single stranded DNA repair mechanism. We are studying novel proteins that assist telomeres with the deprotection response and determining whether these proteins have some other kind of function as well. Telomere deprotection results in chromosomes that are linked together right instead of separate left. I was probably going to have to quit the program or take an out with an easier project. The CIRM internship was very valuable to me. It provided training through stem cell classes and lectures and allowed me to immerse myself in a real lab that had real equipment and personnel. The experience took my research knowledge to the next level and then some. And I knew for sure it had when I was at the poster session during the Bridges conference. I was walking around and asking students about their research, and I understood clearly the path of their research. I knew what questions were good to ask and what the graphs meant without having to take them home and dissect them. I am so excited to start my PhD in the fall. For the first time, I feel confident about my foundational biology and research skills. I also have a better understanding of myself and where I need to improve in comprehension and technique. I am ready to jump into grad school and improve as a scientist. What are your future career steps? I want to do something that involves teaching or being able to educate people. Being a scientist forces you to never be complacent in what you understand. And I see my Professor at Scripps: Do you have advice for future Bridges students? After you start a PhD, you hit the ground running. Usually people who go into PhD programs are people that have always done well in school. A PhD relies on a little bit of luck, getting the right project, and doing everything meticulously. What are your hobbies? My favorite hobby is improv comedy. What I really like about improv is that it is so different from science and it helps me to relax after work. Improv is performing comedic scenes on stage with a bunch of people without a script. You also need to take big risks and not worry so much about what the end result is going to be, which is very different from research. All of my friends outside of work are in improv. In improv we teach a philosophy that everything you have is enough. Everything you come in with is enough. Do you see yourself combining your passions for science and improve in the future? Improv is so important in communication and interpersonal connections. I believe everyone in science could benefit from it. Ideally, I will find a career that allows me to use both of these passions to help people.

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6: Induced pluripotent stem cell - Wikipedia

Telomere shortening and chromosomal instability abrogates proliferation of adult but not embryonic neural stem cells.

Advanced Search Abstract Recently, several groups described the isolation of mouse spermatogonial stem cells SSCs and their potential to develop to embryonic stem cell ESC -like cells, so-called multipotent germline stem cells mGSCs. We show that undifferentiated maGSCs are very similar to undifferentiated male ESCs with regard to global DNA methylation, methylation of pluripotency marker gene loci, telomerase activity and telomere length. Imprinted gene methylation levels were generally lower in undifferentiated maGSCs than in undifferentiated male ESCs, but, compared with undifferentiated mGSCs derived by other groups, more similar to those of male ESCs. Differentiation of maGSCs increased the methylation of three of the four analyzed imprinted genes to almost somatic methylation patterns, but dramatically decreased global DNA methylation. Our findings further substantiate the pluripotency of maGSCs and their potential for regenerative medicine. However, the therapeutic use of ESCs could be impeded by problems regarding immune rejection due to genetic differences between the patient and the donor ESCs as well as ethical issues associated with the use of embryos Vats et al. Spermatogonial stem cells SSCs are self-renewing single cells located in the periphery of the seminiferous tubules whose continuous division maintains spermatogenesis throughout the life of a male individual Spradling et al. Similar to ESCs, these mGSCs could be differentiated into various somatic cell lineages, produced teratomas in immunodeficient mice and formed germline chimaeras after microinjection in blastocysts. Although Kanatsu-Shinohara et al. The two following studies by Seandel et al. Furthermore, another recent study by Kanatsu-Shinohara et al. The molecular processes underlying this fortuitous reprogramming event have not yet been identified. The mammalian epigenome, which is defined as the sum of potentially heritable DNA and histone modifications in a given cell type, undergoes dramatic changes during germ cell development and shortly after fertilization Reik and Walter, ; Li, ; Bernstein et al. In primordial germ cells PGCs representing the precursors of SSCs and all other germ cells, the genome is demethylated and, in particular, the genomic imprints, i. In the mouse, this wave of genome-wide epigenetic reprogramming starts between day In the male germline, the establishment of novel methylation marks for imprinted genes begins around day After fertilization, a second wave of genome-wide epigenetic reprogramming takes place in which the vast majority of male and female germline-derived methylation patterns are erased again and new somatic methylation patterns for development of the new organism are established Mayer et al. To the extent of present knowledge, imprinted genes escape this second wave and maintain their germline-specific methylation and parent-specific expression patterns throughout further development Morgan et al. Thus, imprinted genes display a differential methylation of their parental alleles and maintenance of genomic imprinting in both ESCs and somatic cells. In contrast, pluripotency marker genes such as Oct4 and Nanog switch from a transcriptionally active and demethylated state in ESCs to a transcriptionally repressed and fully methylated state in somatic cells Okita et al. The telomere consists of DNAâ€”protein complexes containing DNA repeat sequences and is essential for protecting chromosomes against degradation and rearrangement Blackburn, Telomerase activity is present in immortal cells such as cancer cells, germ-line cells and ESCs, but not detectable in most somatic cells. The critical role of telomerase activity in male germ cells is underlined by the gradual loss of spermatogenic cells and eventual sterility of mice lacking the telomerase RNA component Lee et al. The possibly germ cell-specific epigenome, in general, and germ cell-specific genomic imprinting, in particular, of mGSCs compared with ESCs was repeatedly discussed as a major obstacle to the potential application of mGSCs in cell substitution therapy Hochedlinger and Jaenisch, To clarify this discussion and test the similarity of maGSCs and ESCs also at the epigenomic level, more systematic studies are warranted. In the present study, we therefore comparatively analyzed the methylation status of several imprinted gene differentially methylated regions DMRs H19, Igf2r, Meg3 and Snrpn and promoter regions of pluripotency-marker genes Nanog and Oct4 , the global DNA methylation, as

well as the telomerase activity and telomere length of undifferentiated and differentiated mouse maGSCs and male ESCs from different genetic backgrounds. We show that the maGSCs and male ESCs are very similar with regard to telomerase activity, telomere length and methylation of pluripotency marker genes. In brief, testis of adult mice was digested using a two-step enzymatic digestion protocol with collagenase and trypsin. After 5â€”10 days in culture, ESC-like colonies could be observed. Cells were cultured for 20 days before DNA was isolated. Pyro Q-CpG software Biotage was used for pyrosequencing data analysis. We employed a recently described modified LUMA protocol for minimizing degradation effects on quantification by performing additional measurements of free DNA ends Bjornsson et al. Results Methylation patterns of imprinted and pluripotency marker genes With regard to methylation of imprinted genes, the hypothesis was tested that undifferentiated maGSCs display somatic imprinting patterns i. For all four imprinted gene regions, the four maGSC lines were generally less methylated showing moderate to strong hypomethylation than the four ESC lines displaying the expected somatic imprinting patterns Fig. For each cell line, we also calculated a mean imprinted gene methylation percentage by averaging the methylation percentages measured for the four imprinted genes Fig. Strikingly, maGSC Stra8 and ESC Stra8, which are derived from the same genetic background, showed very similar mean methylation percentages and moderately hypomethylated patterns for all four tested imprinted gene regions Fig. For other maGSCs and ESCs of the same genetic background, no similarities in mean methylation percentages and methylation patterns were discovered Fig.

7: Telomerase reverse transcriptase - Wikipedia

Telomerase is expressed during embryonic development and in the stem cell compartment of several adult tissues (Blasco, a, Flores et al., ; a; Liu et al.,); however, telomerase levels in these tissues are not sufficient to prevent progressive telomere shortening with age both in humans and mice (Harley et al., , Flores et al.,).

8: Telomeres and telomerase in adult stem cells and pluripotent embryonic stem cells.

telomeres & telomerase in adult sc s & pluripotent embryonic sc s The fact that telomerase activity is largely restricted to SC, suggests that telomerase levels in these cells may be determinant for organism fitness.

9: Telomere Length Reprogramming in Embryos and Stem Cells

Immunofluorescence analysis of pluripotent markers Nanog (red) and TRA (green) in human induced pluripotent stem cells derived from skin fibroblasts. DNA is shown in blue.

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