

Stem Cells: Links to Human Cancer and Aging

The human body develops from a single diploid cell called a zygote and contains at adulthood an estimated 85 trillion cells, of which more than 150 billion turn over every day. All of these cells originate from a tiny population of so-called “embryonic” and “adult” stem cells which uniquely possess a long-term self-renewal capacity and have the potential to differentiate into a variety of cell lineages. “Embryonic Stem Cells (ESC)” is a term commonly used to refer to a distinct cluster of pluripotent stem cells found in the inner cell mass of mammalian blastocysts (early-stage embryos). Their primary function is to give rise to cell lineages of all three germ layers. On the other hand, “Adult Stem Cells (ASC)” is one of several terms used to describe a diverse group of multipotent stem cells clustered in various niches throughout the body, particularly in loci with high cell turnover such as bone marrow, skin, and intestine, but also in sites with low cell turnover such as brain and pancreas. ASC, also known as somatic or tissue-specific stem cells, serve as a renewable source of specialized cells for tissue development, maintenance, and repair. Depending upon the prevailing conditions in their microenvironment, individual stem cells express distinct cell-surface proteins and display differentiation patterns which normally suit the needs of the tissue or organ in which they reside. As discussed later, such stem-cell specialization is enabled by a battery of epigenetic regulatory factors which provide the means not only to arrest and maintain a particular stem-cell behavior, but also to modify it in response to changes in the cell’s microenvironment. Therefore, al-

though ESC and the seemingly various kinds of ASC display different gene expression and differentiation patterns, it remains unclear whether these dissimilarities reflect different cellular entities or different manifestations of the same cellular entity.

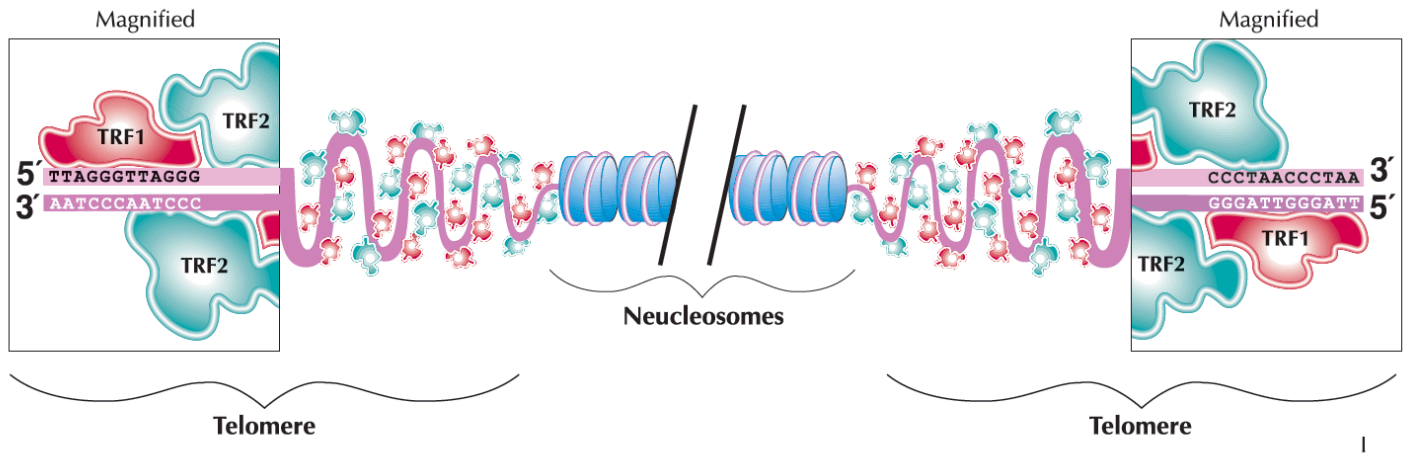
Longevity of self-renewal distinguishes stem cells from their progeny

Stem cells divide infrequently and tend to form and stay within distinct-size clusters. Detachment of cells from the cluster (e.g. as a result of differentiation) triggers rapid replication of the remaining cells until the original cluster size is restored. Upon differentiation, stem cells give rise to rapidly propagating transient progeny, which then differentiate into immature tissue blastocytes. A successive series of proliferating progenitors, displaying steadily increasing lineage commitment, ultimately results in a large number of different mature cells. The distinction between stem cells and their progeny is based on the longevity of their self-renewal, which is commonly assessed by the number of times that the cells can be sub-cultivated in culture conditions before turning senescent (i.e. remain viable but unable to divide). However, since these *in vitro* assays may not reflect the *in vivo* actuality, the term “stem/progenitor cells” is often used to refer to primary cells that could be expanded multiple times in culture while maintaining their multilineage potential.

Telomeres play a key role in determining replicative lifespan

The replicative lifespan of an individual pri-

Figure 1A - Schematic Illustration of a Eukaryotic Chromosome.



many cell depends on the mitotic history of the cell and its ability to maintain functional telomeres at both ends of all chromosomes. Telomeres comprise the ends of each eukaryotic chromosome and consist of several kilobases of repetitive non-coding DNA sequence associated with specialized proteins called telomeric repeat binding factors (TRF1 and TRF2) (Figure 1A). The presence of sufficiently long telomeres at chromosome termini enables complete replication of coding sequences and confers chromosomal stability by reducing the vulnerability of linear DNA ends to nucleolytic degradation and non-homologous end joining. The conservation of the telomere sequence -TTAGGG-n in vertebrates, including bony fish, reptiles, amphibians, birds, and mammals, underscores the importance of telomere structure for genomic integrity and species survival.

Telomere-dependent cellular senescence is a tumor suppressor mechanism

Telomeres shorten with each round of cell division and if not re-elongated ultimately become too short to provide chromosome stability. The presence of such telomeres, called critically short telomeres, constitutes a signal for growth control mechanisms to elicit replicative senescence, a viable cellular state from which no further cell division can occur. Telomere-dependent cellular senescence, which shortens the replicative lifespan of hyper-proliferating cells, appears to be an evolutionary conserved tumor suppressor mechanism and a genetic program for organismal aging. The inherited telomere length in humans (5-15 kilobases) is a genetic

variable that can significantly affect lifespan and the onset of age-related tissue degeneration.

Telomerase maintains telomere length

Homeostasis of telomere length, a hallmark of stemness and cancer, is usually accomplished through the action of telomerase, a multi-component enzyme with reverse transcriptase activity that can counteract replication-induced telomere shortening by re-incorporating lost telomeric segments (50-150 bp/cell-cycling round). The critical components sufficient for *in vitro* expression of human telomerase activity are hTER (human telomerase encoded RNA), which serves as a template for TTAGGG synthesis, and hTERT (human telomere reverse transcriptase) which catalyzes DNA synthesis from the RNA template. In contrast to the constitutive expression of hTER in most somatic cells, significant expression of the catalytic subunit hTERT normally occurs only in germ cells during cell proliferation and in certain subsets of B and T lymphocytes following antigen activation. Downregulation of telomerase activity through repression of hTERT expression appears to occur during early differentiation events associated with mature somatic cell development. Re-activation or introduction of the hTERT gene is sufficient to bypass replicative senescence and to confer cell immortalization, which, if accompanied by inactivation of tumor suppressor genes and the activation of cellular oncogenes can result in neoplastic transformation. It should be noted, however, that although most tumors use telomerase to maintain telomeric DNA, sarcomas often use a telomerase-independent

mechanism called Alternative Lengthening of Telomeres (ALT). The importance of telomerase for stem cell function is highlighted by a rare premature aging disorder called dyskeratosis congenita, an autosomal dominant disorder associated with premature death, typically from bone marrow failure (1). Patients with this disorder have defective telomerase and very short telomeres as a result of germ-line mutations in the genes encoding hTER or hTERT, or as a consequence of dysfunctional DKC1 (dyskerin), a hTER stabilizing protein.

Role of p16 in cancer and aging

Age-related decline in the regenerative potential of tissue-specific stem cells, as a result of changes in their supporting niches, telomere shortening, and other genetic alterations, has been implicated in a number of aging syndromes including graying and loss of hair, osteoporosis, decreased spermatogenesis, fibrosis, and senility. Studies have demonstrated that age-dependent decline in the function of stem cell compartments is associated with increased p16/INK4a-mediated replicative senescence, and that this mechanism is essential for preventing neoplastic transformation of genetically-altered and/or epigenetically-stressed primary cells. The execution of this mechanism is controlled by elaborate epigenetic machinery that tightly regulate transcriptional activation of the INK4a/ARF locus, a chromosomal site (9p21 in humans) comprising overlapping reading frames encoding two independent cyclin-dependent kinase (CDK) inhibitors, p16/INK4a (p16) and p14/ARF (ARF). The latter is a positive regulator of p53, a tumor suppressor protein that in addition to triggering apoptosis in response to DNA damage, can also elicit cell-cycle arrest in hyper-proliferating cells even in the absence of DNA damage. Interestingly, critically short telomeres associated with TRF2 are resistant to p53-mediated apoptosis, whereas telomeres lacking TRF2 protection trigger length-independent apoptosis by both p53 and ATM (Ataxia Telangiectasia Mutated gene product) pathways (2). The p53-dependent cell cycle arrest is reversible upon subsequent inactivation of p53, while the replicative senescence induced by p16, either by itself or

through activation of retinoblastoma proteins (pRB), is permanent and appears to result from the irreversible formation of repressive heterochromatin at loci containing a number of cyclin and CDK genes. Cyclin-CDK complexes regulate progression through the cell cycle by inactivating, through phosphorylation, retinoblastoma proteins, which are key regulators of cell proliferation and differentiation during normal development and after genotoxic stress.

The expression of p16 is induced by a variety of stressful stimuli including expression of oncogenes, suboptimal culture conditions (or niche support), and loss of polycomb repressive complexes. Polycomb repressive complexes are evolutionary conserved chromatin regulators that silence target genes through a variety of different mechanisms (3). They have been shown to regulate developmental genes in multiple cell types and are widely recognized for their essential roles in embryonic development and somatic cell reprogramming (4). However, the presence of these complexes at the p16/ARF locus of stressed cells has been implicated in cancer (5). The importance of a functional p16/ARF locus for preventing tumor development is underscored by the high frequency of mutation of this site in human cancers. The relevance of this site for tissue aging has been suggested through a series of studies showing that single nucleotide polymorphisms near this locus are associated with age-related pathologies including frailty, Type 2 diabetes mellitus, and late onset Alzheimer's disease (reviewed in ref. 6).

The p16/ARF locus is epigenetically repressed in early life and then subjected to progressive activation, resulting in steadily increasing levels of p16 with age. The demonstration that p16 levels accumulate in stem cells of old mice suggests that these levels can constitute a good overall biomarker for aging (7). Age-dependent expression of p16 in stem cell compartments is associated with widespread tissue degeneration, whereas deficiency of p16 expression increases tissue regenerative potential accompanied with tumorigenesis (reviewed in ref. 7). These observations suggest that cancer prevention by p16-mediated cellular senescence

might come at the expense of accelerated tissue aging. This notion is further supported by studies on the link between p16 and ID-1, a helix-loop-helix protein that can specifically inhibit p16 expression but not that of ARF. ID-1 is a potent inhibitor of cell differentiation and plays an important role in the maintenance of many mammalian primary cells by coordinating cell division and differentiation. However, it has been shown that ID-1 can also promote cancer development by stimulating the proliferation, invasion, and survival of several types of human cancer cells. High expression of ID-1 has been observed in a large number of cancers including prostate, breast, ovary, thyroid, colorectal, liver, pancreas, and other tumors. Constitutive expression of ID-1 in cultured human primary melanocytes extends their lifespan in association with decreased expression of p16 but without notable changes in cellular growth, migration, or telomere length. In contrast, ID-1-null primary mouse embryo fibroblasts undergo premature senescence associated with increased expression of p16 but not ARF (8).

Epigenetic Remodeling of Chromatin Structure

The versatility of individual stem cells to respond to diverse external cues by selecting and executing a suitable genetic program out of many permissible ones, or their epigenetics, enables a single and otherwise fixed genome to yield a plethora of cell types. Although the mechanisms underlying stem cell identity and plasticity have not been fully characterized, it has become clear that epigenetic remodeling of chromatin structure, particularly through covalent modification of genomic DNA and cognate histones, is pivotal in the establishment and maintenance of cellular identity, memory, and potentiality.

Chromatin undergoes numerous modifications

The basic repeating unit of chromatin, termed nucleosome, consists of a 146 base-pair DNA chain wrapped around a histone octamer made of two of each of the four core histones, H2A, H2B, H3, and H4. In higher-order chromatin structures, the nucleosomes are connected via DNA linkers of varying lengths coupled with histone H1 (Figure 1B). Core histones are evolutionary conserved, structurally related proteins containing a highly positively charged, N-termi-

Figure 1B - Schematic Illustration of un-modified (a) and modified (b) nucleosomes.

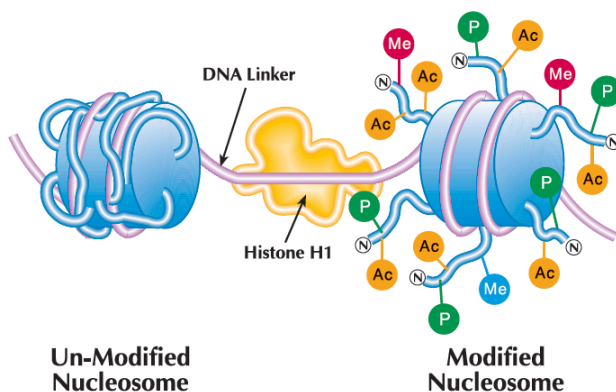


Figure 1D - Schematic Illustration of euchromatin (left) and heterochromatin (right).

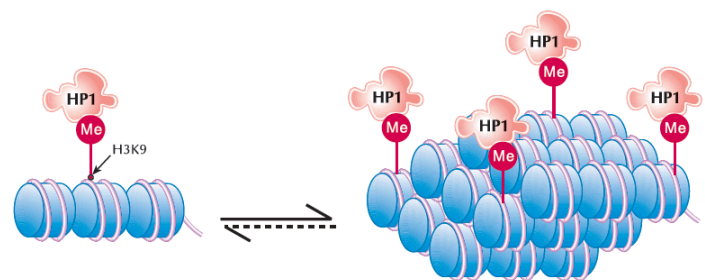


Figure 1C - A heavily modified Histone H3 tail (an arbitrary example).



nal tail of 25-40 residues that extends through the DNA coils and into the space surrounding the nucleosome. Nucleosomal histones, in particular their exposed tails, are subject to various post-translational modifications including methylation of lysine (K) and arginine (R) residues, acetylation and ubiquitination of K residues, and phosphorylation of serine (S) and threonine (T) residues (Figure 1C). Nucleosomal DNA is subject to specific epigenetic methylations, particularly of cytosine moieties within CpG dinucleotides. The functional relevance of the various covalent modifications of chromatin, which can be reversed through the action of specific nuclear enzymes, such as demethylases, deacetylases, and phosphatases, is a topic of current research and will be discussed here in general terms only.

Chromatin modifications are linked to aging and cancer

Post-translational modifications of core histones trigger specific alterations in the spatial organization of chromatin, which in turn affect DNA-based processes including DNA repair, transcription, replication, and recombination. In the absence of histone modifications, the positively charged tails of core histones form stable salt bridges with negatively charged inter-nucleotide phosphate groups of adjacent DNA (Figure 1B). Such interactions can prevent, for example, interaction of RNA polymerase with promoter regions of genes whose expression is not needed by the cell. Modifications that reduce the net positive charge of histone tails, such as acetylation of K residues (neutralizes their positive charge) and phosphorylation of S and T residues (rendering them negatively charged), weaken tail-DNA interactions (Figure 1B) and are generally associated with transcriptionally active genes. Certain modifications, particularly methylation of specific K and R residues, generate docking sites for nuclear proteins that are involved in activation or repression of specific gene loci. For example, methylation of the K residue at position 4 of histone H3 produces binding site, H3K4Me*, for the chromatin remodeling protein Chd-1. Subsequent to its binding to H3K4Me*, Chd-1 recruits nucleosome remodeling enzymes such

as acetylases and phosphokinases, whose action positively regulates DNA replication by disrupting H3 tail-DNA interactions. In contrast, the heterochromatin-associated protein HP-1 interacts with H3K9Me* and promotes formation and maintenance of replication-incompetent heterochromatin (Figure 1D). The symbol [Me*] denotes K residues that are either mono-, di-, or tri-methylated. Interestingly, high-resolution profiling of histone methylations in the human genome has revealed that tri-methylated H3K27 signals were higher at silent promoters than active promoters, whereas an opposite trend was associated with mono-methylated H3K27 (9). Tri-methylation of this residue, which produces H3K27Me3, is catalyzed by the polycomb repressive complex (PRC)-2 which contains the histone methyltransferase EZH2. H3K27Me3 serves as a docking site for the bulky, BMI1-containing PRC-1, whose presence at this site blocks accessibility to many gene loci including the p16/ARF locus (5). As already mentioned, sustained repression of the p16/ARF locus, which requires constitutive expression of PRC components such as EZH2 and BMI1 (an oncogenes), has been implicated in the development of various cancers. On the other hand, decreased expression of PRC components, particularly EZH2, in older or stressed cells has been suggested to be a major cause for the steady increase in p16 levels with age (5). Also, since p16 is a phosphokinase inhibitor, it is possible that p16-mediated inhibition of histone phosphorylation at the promoter region of EZH2 or other PRC components indirectly contributes to its own expression. Likewise, P16-induced cellular senescence may result, in part, from p16 inhibition of histone phosphorylation at nucleosomes whose DNA transcription is required during mitosis and dependent upon such phosphorylation.

Therapeutic targeting of the Epigenome

Advances in the development of analytical methods for determining histone methylation profiles across large genomic sequences have enabled closer examinations of the so called “epigenetic signature” or “epigenome” of somatic cell types (9, 10). The general picture emerging from these studies is that methylation

of specific K and R residues of core histones is a fundamental mechanism for establishing and maintaining gene expression patterns that can carry epigenetic information through cell division. The epigenome of Embryonic Stem Cells (ESC) has been found to be enriched for chromatin structures displaying histone methylation marks of both transcriptionally active and silent promoters (10). These chromatin structures, called “bivalent domains”, have been suggested to comprise transcriptionally repressed chromatin that is poised for selective activation by, for example, differentiation-inducing signaling pathway intermediates (10). Global silencing of developmentally important genes that can be selectively activated in response to environmental cues appears to be controlled by a small group of transcription factors (11-13). For example, it has been shown that retrovirus-mediated introduction of four transcription-factor genes, Oct4, Sox2, c-Myc, and Klf4, into adult fibroblasts selected for nanog expression was sufficient to confer a pluripotent state upon the fibroblast genome (12). Analysis of the reprogrammed epigenome of such induced pluripotent cells revealed that it was almost indistinguishable from that of ESC (13). These results provide direct evidence that all chromatin modifications are reversible and support the notion that targeted manipulation of the epigenome by agents that can induce reorganization of chromatin is a viable approach for the discovery of new therapeutic drugs for cancer treatment. Indeed, the number of drugs targeting epigenetic modulators has increased significantly in recent years (14). Likewise, it should be interesting to determine the effects on the epigenome of dietary and physical regimens which appear to prolong lifespan or reverse the course of age-related pathologies, such as caloric restriction and boxing workout by Parkinson’s patients, respectively.

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