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review

Stem cells, DNA damage, ageing and cancer

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A comprehensive knowledge of adult stem cells not only sheds light on their significant roles in many fundamental biological process, but also on their implications in a variety of deleterious disorders including leukemia, lymphoma, ageing and other relevant degenerative disorders. Elucidating the diverse signaling pathways in stem cells paves the way for development of targeted therapeutic approaches against degenerative disorders. Despite emerging studies, our understanding of the key molecular machineries that profoundly influence key stem cell properties is poor. This review discusses the seminal aspects of stem cell self-renewal mechanisms with an emphasis on various influencing factors, including DNA damage, and intrinsic and extrinsic factors that govern the significant characteristics of tissue stem cells under normal and stress conditions. Also outlined are intriguing aspects of how molecular interventions in key stem cell pathways could be exploited to develop novel therapies against stem cell disorders.

Adult stem cells are a life-time reservoir for various lineage-specific cells and are responsible for remarkably preserving both tissue function and capability to respond against various stresses. Since mature blood cells are predominantly short lived, hematopoietic stem cells are required throughout the lifespan to replenish multi-lineage progenitors and precursors committed to their hematopoietic lineages. Generally, adult stem cells are endowed with unique self-renewal and differentiation abilities to generate their daughter stem cells and mature cell types, respectively. Recent evidence suggests that although primitive, long-term reconstituting hematopoietic stem cells (LT-HSCs) remain relative quiescent. They can reversely and infrequently switch into cell cycle to self-renew or differentiate into multi-lineage progenitor cells.[1] In the hematopoietic hierarchy system, LT-HSCs divide infrequently to supply more proliferative short-term HSCs, which in turn generate various multi-potent progenitors that as a consequence, give rise to more differentiated progenitor cells.[2] Under homeostatic conditions, stem cells continuously self-renew and differentiate with only limited proliferative potential so as to protect the hematopoietic cells from mutagenic insults, ageing and carcinogenesis. The striking balance among relative-quiescence, self-renewal and multi-lineage commitment is regulated by various intrinsic and extrinsic regulatory mechanisms (Figure 1).[3]

Molecular features of self-renewal in stem cells

A defining characteristic of stem cells is their impressive self-renewal potential with long-term differentiation capabilities. The fascinating aspect is that stem cells of the same tissue origin can show remarkable differences in their self-renewal and cell cycle characteristics at different developmental stages. Embryonic stem cells (ESCs) derived from the inner cell mass of the early mammalian embryo have pluripotent potential, with the ability to differentiate into virtually all the cell types of an adult animal. In contrast to ESCs, adult stem cells possess only multipotent potential giving rise to differentiated and lineage-committed tissue cells. Although HSC are evolved from ESC, they migrate through different organs during developmental stages with the specific purpose to replenish tissue maintenance and repair. In early embryogenesis (period of 11.5-16.5 embryonic days), HSCs are driven to the fetal liver for profound expansion and then eventually migrate to the bone marrow niche where they remain under relative quiescence, but infrequently undergo self-renewal and a multipotent proliferation process.[4] This demonstrates the adoptive nature of stem cells corresponding to their developmental requirements. For instance, fetal HSCs were shown to have an intrinsic ability to self-renew and differentiate, which was maintained until 1 week after birth. At this time, the HSCs switched to being quiescent, similar to adult HSCs. For instance, it is apparent that the self-

renewal characteristics of stem cells remain active not only during early development, but also in adult stem cells for the purpose of development of mature organs.[5,6] Moreover, ESCs undergo symmetrical self-renewal divisions, with one stem cell giving rise to two daughter stem cells, resulting in an expansion in stem cell numbers. In contrast, adult HSCs predominantly undergo asymmetrical self-renewal divisions, generating one stem cell and one more committed cell, thus preserving stem cell numbers while enabling blood cell regeneration in vivo.[7] HSC undergo symmetrical self-renewal divisions only in specific and temporally restricted developmental contexts. ESCs lines could be maintained and expanded in vitro for a longer time period whereas adult HSCs have limited in vitro potential. Interestingly, the fate of ESC is more stringently regulated and less susceptible to quiescence and senescence than HSC.

Stem cell functionalities are profoundly regulated by complex mechanisms arising from cell-autonomous regulation, to their local and systemic milieu within the stem cell niche, the surrounding tissue, and the external environment. Alteration cues from any or a combination of these signals could influence the functionality and/or number of stem cells. Self-renewal is activated by diverse signals, such as developmental regulators or certain oncogenes. Studies report that self-renewal is linked to the cell cycle.[6] The regenerative potential of stem cells is restricted in such a fashion they can only replicate a definite number of times during the lifespan of an organism, suggesting an intrinsic limitation of the stem cell. This intrinsic limitation is attained if stem cells are induced to proliferate rapidly due to environmental stresses such as radiotherapy or chemotherapy.

Intrinsic mechanisms of self-renewal in stem cells

Although stem cells accrue DNA damage over the lifetime of an organism, studies illustrate that both stem cell frequency and absolute numbers are not profoundly

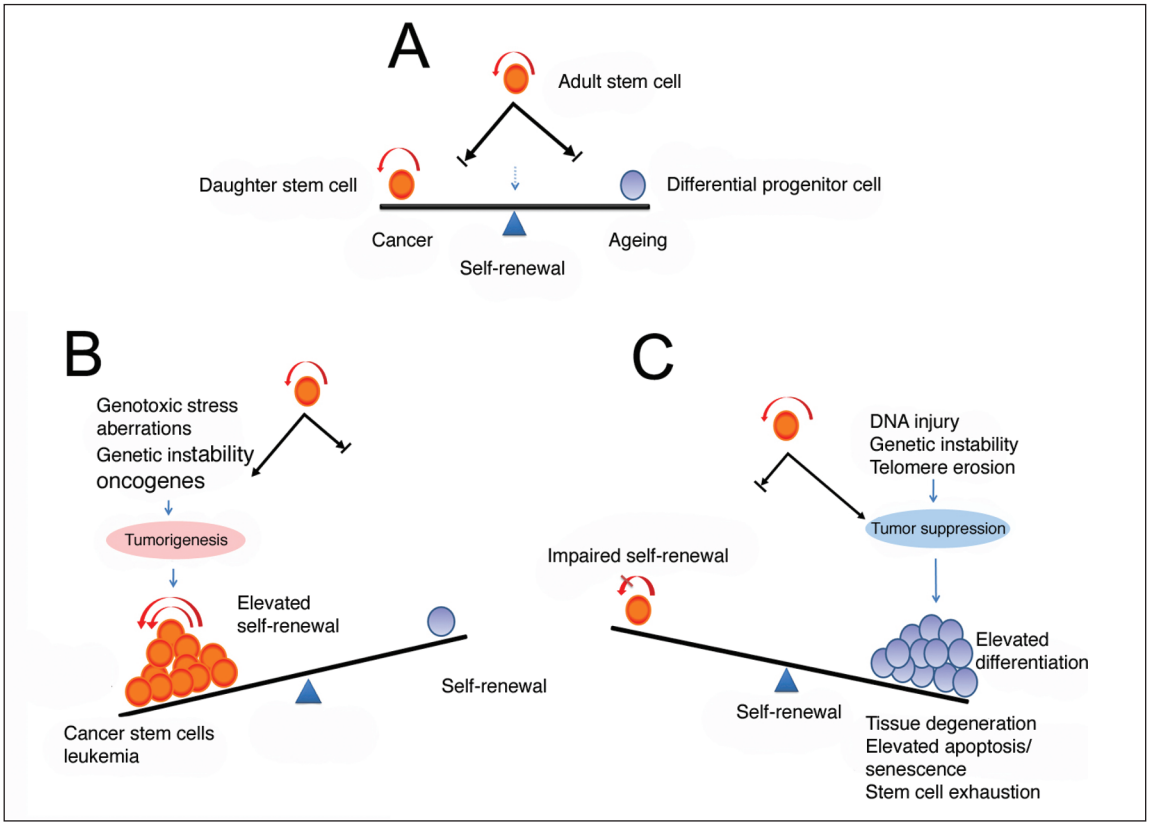

Figure 1. The physiological consequences of balance and imbalance self-renewal processes in stem cells (self-renewal represented by the counterclockwise arrow over the adult stem cell). (A): delicate balance between self-renewal and differentiation properly maintains the stem cell number with regular homeostasis, tissue function and tissue repair. (B): elevated self-renewal triggered by various factors initiate tumorigenesis with profound increase in stem cell numbers which leads to cancer formation. (C): impaired self-renewal reduces the size of the stem cell pool and increases the stem cell differentiation thereby leading to tissue degeneration, stem cell exhaustion, and ageing phenotype.

diminished under normal conditions.[8] Similarly, their interaction with stroma is not diminished with age. However, interestingly, HSCs are less effective at homing and engrafting, suggesting that intrinsic characteristic of HSCs could play a role in age-mediated attrition of HSCs functionalities as DNA damage accumulates. Most of the developmental pathways involved in self-renewal are conserved in many tissues and in many organisms. Although key master regulators of adult HSC cell fate are still poorly defined, the molecular bases of ESC self-renewal are more rapidly unfolding, with recent evidence suggesting the involvement of distinct set of genes viz., Nanog/Oct4/Sox2 and Tbx3/Tcl[1,9] in the regulation of separate pathways. Studies elucidate that Notch, Wnt and Hedgehog-mediated fundamental mechanisms commonly exhibit self-renewal machineries by either activating or repressing many target genes involved in diverse stem cells population from hematopoietic, neural tissue, skin, epithelium and germline tissues.[6] Recent investigations suggest that several pathways exhibit a self-renewal process.[5] For instance, WNT proteins, Hedgehog proteins, bone morphogenetic proteins (BMPs) and the Notch stimulate the activation and translocation of transcription factors into the nucleus.[3] In this, Notch activation is critical to maintaining the self-renewal of stem cells in diverse stem cell niches from the worm germ line to mammalian HSCs. One common mechanism by which stem cells maintain self-renewal is inhibition of differentiation. This is apparent in both ESCs and tissue stem cells where Foxd3, the winged-helix transcription factor is reported to suppress the differentiation and thereby maintain the self-renewal in both pluripotent and multipotent stem cells.[10-12] The self-renewal process should be stringently used to correspond to tissue homeostatic demands. Continuous activation of the self-renewal program can lead to stem cell exhaustion. For instance constitutively activated β-catenin leads to prominent defect in multilineage differentiation.[13,14]

Although some critical self-renewal pathways commonly drive the downstream signaling machineries in most stem cells types, some pathways are unique to certain tissues or under specific circumstances. Concerted and stringent regulation of multiple pathways tightly regulate the self-renewal program in ES cells, which are unique to pluripotent cells. The molecular switches that govern global transcriptome machineries are differently regulated while transiting through various developmental stages.[15] For instance, Oct4[16] and Nanog[17] are transcription factors required for pluripotency during early embryogenesis and for maintenance of ESC identity, but not essential for multipotentiality in somatic stem cells. Fetal HSCs require Sox[18] for the maintenance whereas it is dispensable in adult HSCs. Conversely, Gfi-1 and Et6 are essential for self-renewal in adult HSCs but not in fetal HSCs. A recent study confirms that Zfx, a transcriptional regulator mediates self-renewal in both embryonic and adult stem cells, suggesting a common mechanism for self-renewal.[18] Interestingly, deletion of Zfx impaired self-renewal, but not the differentiation capacity of murine ESC; conversely, Zfx overexpression facilitated ESC self-renewal by opposing differentiation. Furthermore, Zfx deletion abolished the maintenance of adult HSC, but did not affect erythromyeloid progenitors or fetal HSC.[18] Taken together, these reports confirm that stem cells employ different mechanisms at various developmental stages to regulate the pluripotency as compared to multipotency irrespective of some shared molecular programs.

As the self-renewal process tightly couples with cell cycle regulatory pathways, cell cycle signaling cascades dictate self-renewal and proliferation in tissue stem cells. Cyclin D-CDK4/6 plays a prominent role in entry into cell cycle by inactivating Rb proteins, the mediator of quiescence. D-cyclins are essential for the expansion of hematopoietic stem cells by enhancing their self-renewal and their absence is exemplified by stem cell depletion during fetal development,[19] but they are dispensable in ES cells. Ink4 family proteins negatively regulate self-renewal by down-regulating D-CDK4/6 complex and also reduce both the frequency and function of stem cells. Likewise other tumor suppressor machineries for instance, p19/p53/p21 pathway negatively regulates stem cell frequency. Depletion of these factors enhances stem cell frequency and functionality. For instance, Bmi-1, a repressor of p16Ink4a/19Arf promotes self-renewal in the postnatal period. Deficiency of Bmi-1 profoundly decreases self-renewal and depletes the stem cell pool in various tissues.[20] Collectively, these studies illustrate a tight regulation of self-renewal machineries which profoundly maintain a perfect balance to meet the regenerative demand throughout life, while avoiding extensive transformation into cancer (Figure 2).

Transcription driven self-renewal

Interestingly, the switch between self-renewal and differentiation is also dictated by competitive actions of transcription factor complexes. For instance, the transcription factors, GATA1 and PU.1 mediate the erythroid and myeloid lineages, respectively. They inhibit each other's ability to bind to the target genes such that if GATA1 is deficient, PU.1 shifts the balance toward the myeloid program. Conversely, an absence or deficiency of PU.1 leads GATA1 to drive erythroid program.[21-23] Besides, competitive transplantation experiments suggest that

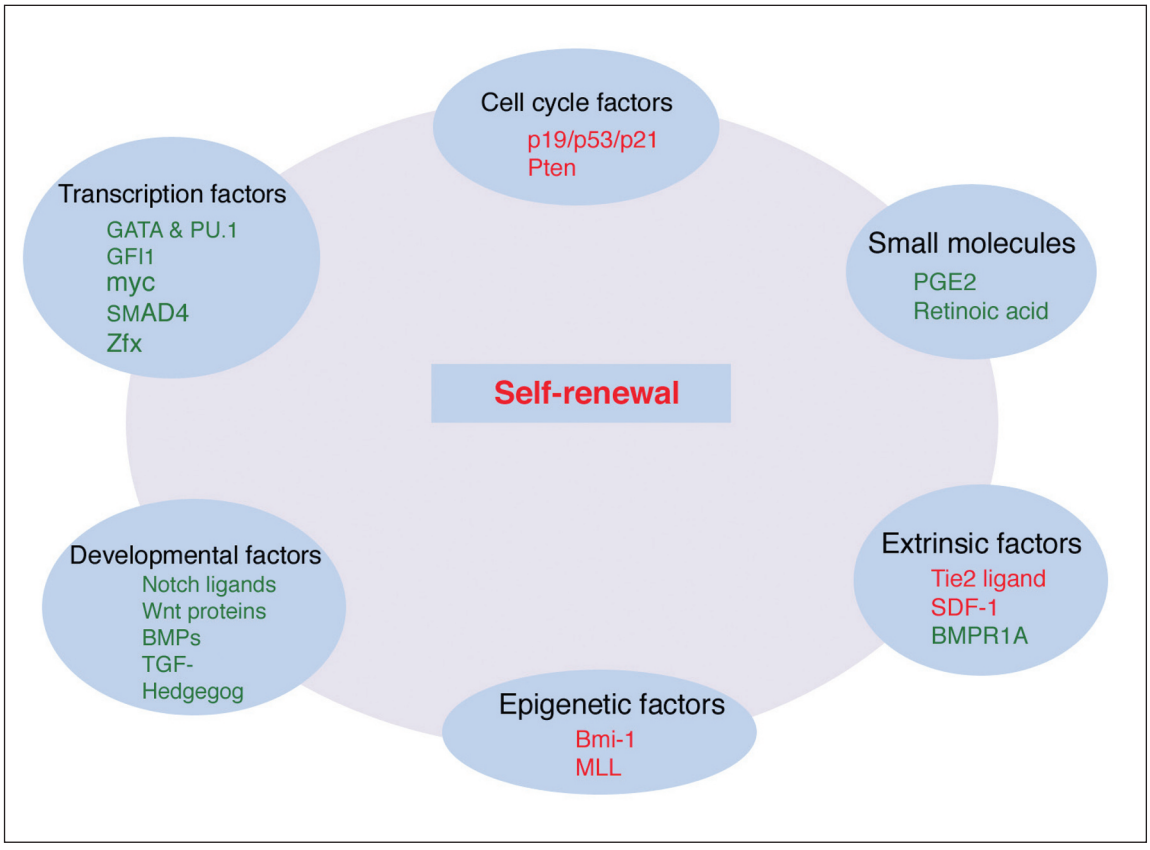

Figure 2. Regulators involved in self-renewal processes in stem cells. Stem cell self-renewal is activated by diverse signals such as developmental regulators, transcription factors, cell-cycle associated factors, small molecules, extrinsic factors, and epigenetic factors via both extrinsic and intrinsic mechanisms. The positive regulators of self-renewal are indicated in green fonts, whereas negative regulators are shown in red fonts. TGF-β, transforming growth factor-β; Zfx, X-linked zinc-finger protein.

down-regulation of transcription factors GATA2, GFI1, myc and SMAD4 inhibit the self-renewal characteristic of HSCs. The impact of the transcription pathways on stem cells is profound, such that a set of transcription factors including OCT4, SOX2, Myc and KLF4 could reprogram a fibroblast to induce a pluripotent stem (iPS) cell, demonstrating that transcription factors can induce pluripotency and self-renewal.[24] Mutation in this transcription machinery could disrupt the homeostatic balance to favor either self-renewal or differentiation.

Role of epigenetic mechanisms in self-renewal

New studies have implicated a role for epigenetic regulation in the self-renewal of hematopoietic and neural stem cells. Interestingly, certain chromatin-associated factors could either silence or activate the gene expression of specific genes by histone modifications that in turn modulate the self-renewal. This phenomenon could be best illustrated by epigenetic mechanisms of Bmi-1, a member of the Polycomb group (PcG), known for promoting self-renewal. BMI-1 actively engaged in the self-renewal program by regulating the transcription of cell-cycle regulator INK4A (a negative regulator of CDK4-D-type cyclins).[17] The defective self-renewal of HSC has been attributed to derepression of $p16^{Ink4a}$ and $p19^{Arf}$ via Bmi-1 targeted silencing mechanism and impaired self-renewal, at least in part, could be reversed by downregulation of these target genes in Bmi-$1^{-/-}$ stem cells. This accounts for why mice with Bmi$1^{-/-}$ mice possess impaired stem cell pool associated with progressive postnatal pancytopenia.[25] BMI1 also shown to repress transcription factor E4F1 in haematopoietic stem cells epigenetically. Similarly, overexpression and inactivation of MLL, regulated by the Polycomb complex, lead to increased self-renewal[26] and deficient HSC self-renewal[27] respectively. Epigenetic changes in stem cells are not permanent and can be erased (partly or completely) by cell division. Therefore, such changes might facilitate the transition of a progenitor cell to a self-renewing stem cell, or prompt differentiation with diminished self-renewal

capacity. Altogether these findings demonstrate the essential roles of epigenetic mechanisms in self-renewal process which could possibly pave the way for developing stem cell therapeutic interventions by exploiting PcG genes as potential target.

Extrinsic pathway modulators-small molecules in self-renewal

Systemically, stem cells are continuously influenced by extrinsic regulation from their hematopoietic-inductive microenvironment or stem-cell niche in order to maintain primitive stem cells in the undifferentiated form. Certain key factors secreted by the bone marrow are suggested to have pivotal roles in regulating the quiescence of stem cells.[28] However, the detailed mechanisms of the extrinsic modulators in the maintenance of the stem cell population remain elusive. For instance, osteoblasts from the stem cell niche release the Tie2 ligand (angiopoietin-1) to mediate quiescence in the stem cell pool. Similarly SDF-1 factor has also been secreted by endosteal cells in order to maintain the HSC pool.[29] Down regulation of BMPR1A, which is normally expressed from osteoblasts, enhances the regenerative potential of HSCs. Signals from distal organs could also influence stem cell maintenance. For instance, diminished signaling from bone morphogenetic proteins in the ovary could lead to a decline in germ stem cells numbers thereby trigger ageing. Studies also confirm that the HSC pool can be linearly increased in accordance with the osteoblast population, suggesting that the niche plays an important role in maintaining a quiescent or primitive HSC population. Studies also indicate that the niche factors also influences mobilization, homing and engraftment of HSCs. For instance CXCL12, a cytokine expressed from bone-marrow sinusoidal endothelial cells (BMECs), largely influences the mobilization, homing and engraftment of HSC.[30]

Several research groups have carried out chemical screening for small compounds that modulate self-renewal in ES cells, neural stem cells and other adult stem cells. The underlying mechanisms by which certain small molecules regulate the self-renewal in stem cells have been studied to characterize the molecular signatures of self-renewal and to develop potential therapeutics. Retinoic acid, for instance, leads to alterations in HOX gene expression during embryogenesis and is a modifier of the WNT-mediated signaling pathway.[31,32] Prostaglandin E2 (PGE2), a small lipid mediator, has also recently been shown to regulate HSC self-renewal during embryogenesis and can enhance HSC engraftment, as measured by the competitive repopulation studies in mice.[33] Self-renewal can be augmented in stem cells with self-renewal potential. HSCs can execute the self-renewal programme, but the addition of WNT3A, sonic Hedgehog (SHH), and angiopoietin-like factors or PGE2 can increase the size of stem-cell pool.

Stem cells under genotoxic stress/DNA damage

Protecting the genome integrity represents a fundamental and continuous challenge to stem cells compared to their progenitors and differentiated cell types. Adult stem cells are susceptible to a variety of genotoxic stresses such as DNA damage and oxidative stress due to their inherent characteristics. First, given the fact that HSCs are maintained throughout the lifespan of an organism, a single HSC could accumulate multiple mutations necessary for transformation. In other words, accumulating mutations are a potential concern not only to the self-renewing stem cells, but also to either daughter cells or downstream progenitor lineages thereby largely compromising the genetic stability in multitude hierarchy levels. Secondly, stem cells share common features of tumor cells, i.e., a high proliferation potential that renders them susceptible to acquisition of multiple mutations. Thirdly, the relative quiescent nature of stem cells renders them resistant to chemotherapy and they are, therefore, a potential source of tumor recurrence. Cells have evolved multiple mechanisms, including several DNA repair pathways, to remove DNA mismatches and lesions and prevent the deleterious consequences of DNA damage. Under relative quiescence, with a low metabolic activity, stem cells could discourage genetic lesions induced by reactive oxygen species (ROS) which otherwise could contribute to DNA injury. Another protective mechanism of HSC comes from their bone marrow niche, which is predominantly hypoxic with low levels of oxidative stress. At the molecular level, stem cells efficiently efflux xenobiotic genotoxic components through the ABC transporter system, which greatly reduces DNA damage. Another interesting mechanism by which stem cells might prevent erroneous genetic lesions could be attributed to asymmetric segregation of genetic information.[34,35] Asymmetric cell divisions yield one daughter stem cell and one committed progenitor cell. This intriguing mechanism allows a parent stem cell to segregate defective genetic information to a progenitor cell while passing the error-free chromosomes to a daughter stem cell. This would tempt us to postulate that through asymmetric division stem cells not only preferentially retain the chromatids with 'active stemness'/stem cell-specific genes, but also retain the template without any replicative errors to maintain high fidelity in stem cell characteristics. However, the magnitude of the aforementioned protective mecha-

nisms against the genetic instability remains to be investigated in detail.

Although, accrual of mutations is regularly checked by tumor suppressor pathways in adult stem cells, continuous homeostatic demands inadvertently compromise tumor suppressor mechanisms and profoundly challenge the genetic stability of stem cells. If DNA damage accumulation exceeds the levels of repair systems or DNA damage response is deficient, unrepaired damages would propagate in the cells. Accumulating DNA lesions in stem cells could be cytotoxic and or cytostatic leading to a large reduction in regenerative potential and homeostatic maintenance as observed in an ageing phenotype. Alternatively, if lesions in stem cells are mutagenic, they could greatly contribute to carcinogenesis. At a given dose of radiation, adult stem cells and their immediate descendants are hypersusceptible to initiation of radiation-induced apoptosis triggered by a DNA damage response. Indeed, this hypersusceptibility in stem cells is a testament to stringent cellular checkpoint programs available to protect the genome integrity. Failure to initiate apoptosis would be detrimental to the damaged tissue stem cells, as the flawed genome with prevalent mutations would descend to their progenitors in a higher magnitude.

DNA damage-induced impairment in stem cell function leads to several clinical syndromes such as diminution in stem cell pools, generation of cancer stem cells inducing apoptosis or it causes differentiation and bone marrow failure. Increased genetic instability in LT-HSCs was shown to compromise the reconstitution functionality.[35] A recent report by Nijnik et al confirms that defects in DNA double strand break repair influence stem cell hematopoietic reconstitution function.[36] Furthermore, a number of DNA repair abnormalities are closely linked to premature aging syndromes with defects in the stem cell population. In most acute myeloid leukemia (AML), HSCs like cells such as $CD34^{+}CD38^{-}$ phenotype cells are reported to have an intrinsic ability to initiate the leukemiagenesis.[37] An interesting study by Graham et al has shown the presence of a significant quiescent (G0) population within the $CD34^{+}$ population of hematopoietic cells from patients with CML.[38,39] In this case, an impaired DNA damage response observed in quiescent HSCs could be explained by the fact that those HSCs under G^{0} phase (quiescent HSCs) are deficient in the homologous recombination repair pathway. A functional impairment in DNA damage repair is the rate-limiting step for the maintenance of stem cells.[36] Accumulating genetic lesions could drive the ageing process.[40] Although stem cells from aged mice with accumulating DNA mutations still could maintain the regenerative potential, their regenerative potential is severely compromised after injury.[8,41] Together, these results are consistent with the view that DNA damage accumulates with ageing in the HSC compartment and that this damage can be physiologically significant if unrepaired. It is therefore how stem cells respond to genotoxic stress that is pivotal in understanding the underlying mechanisms of leukemiagenesis and aging. Nonhomologous end-joining (NHEJ) and homologous recombination (HR) are mechanistically distinct DNA repair pathways that ensure the repair of DNA double-strand breaks (DSBs). Failure of both NHEJ and HR pathways in HSCs could impact both function and proliferation efficiency.[42]

Stem cells in the ageing process

There is growing interest in the scientific understanding of aging, as biological explanations are increasingly paradoxical to the extent that they are largely unknown despite extensive research. After all, mortality is the ultimate destiny for any organism despite survival mechanisms at both the cellular and organism levels. Just as mortality is inevitable-ageing is also inevitable. Our scientific understanding suggests that impaired tissue maintenance and repair are hallmarks of ageing, which is predominantly associated with an age-dependent decline in self-renewal and the multi-potency potential of adult stem cell. Unraveling the molecular mechanisms that drive the stress-mediated decline in stem cell functionalities is pivotal to understand the ageing phenotype. Stem cell depletion due to the accumulation of DNA damage has been reported in animals with genomic instability and has been implicated in the decline of tissue renewal capacity and the appearance of aging-related phenotypes.[8,41] Stem cell depletion is a predominantly triggered cellular defense mechanism via stem cell senescence and/or apoptosis following DNA damage.[43,44] The aging process is tightly driven by various mechanisms such as intrinsic, extrinsic and systemic factors. Such factors exhibit different levels of influence on the ageing process corresponding to the cell phenotype.[45] In this, the impact of extrinsic factors can be exploited, at least in part to reverse the aging process, which was induced externally. For instance, a forced Notch signaling, through an extrinsic mechanism could rejuvenate aged-adult skeletal muscle cells.[46] Further, this study demonstrates that TGF-β/pSmad3 attenuation in old, injured muscle restores regeneration to satellite cells in vivo. However, HSCs also potentially accrue the aging characteristics with reduced self-renewal and altered differentiation capac-

ity, indicating that intrinsic factors must be responsible for the reduced self-renewal capacity. Here, the intriguing possibility could be that the distinct aged HSCs phenotype as a result of their milieu, systemic factors or even intrinsic events might be manipulated to rescue some age disorders.

Generally telomere length, known as a molecular clock, defies chronological ageing to maintain the mortality of cells. Intrinsically, telomerase activity directly impacts the longevity of self-renewing of primitive stem cells.[47,48] The importance of the telomerase activity is illustrated by the fact that germinal cells with profound replicative efficiency in the ovaries and testis display an elevated level of telomerase activity.[49] At the molecular level, low telomerase shortens the telomere to induce telomere dysfunction accompanied by profound DNA damage. This raises an interesting question how quiescent stem cells, despite their low telomerase expression maintain their longevity. Studies show that quiescent stem cells might enhance their TERT, a catalytic subunit of telomerase, thereby maintaining the telomeres and their cell replicative potential.[50]

Telomere dysfunction activates a cascade of tumor suppressor mechanisms initiated by $p16^{INK4a}$ and ARF and thereby up regulates p53/Rb, causing senescence and apoptosis. This is exemplified by the fact that significant up regulation of the senescence-associated β-galactosidase and $p16^{INK4a}$ observed in ageing organisms. It is tempting to speculate that down-regulation of p53 would enhance the self-renewal of tissue stem cells and possibly their reconstitutive potential. However, studies show that down regulation of p53 could rescue a stem cell defect, but inadvertently compromise tumor-suppressor activity, thus leading to a shorter lifespan with carcinogenesis.[51] A striking balance between self-renewal and carcinogenesis could be attained if $p21^{CIP}$ instead of p53 is targeted to extend the stem cell longevity without leading to tumorigenesis in telomere-deficient mice.[52]

Given that behaviors of HSCs are stringently and coordinately regulated, it is inevitable that stress inducers from within and from their systemic milieu can frequently exacerbate their phenotypes. Besides, a recent study confirms that aged HSCs also contribute to the impaired stem cell function and engraft aged hematopoietic environment in telomere dysfunctional $Terc^{-/-}$ mice.[51] Stem cells regularly undergo stress during their lifetime and have evolved to deal with a variety of stresses that diminish their potential. These stressors, including reactive oxygen species, acute or chronic DNA damage, telomere shortening,[53,54] 5-fluorouracil (5-FU), are proven to have an impact on tissue SC functionalities. Generally, chronic stress leads to decline in HSC number and the function of HSCs mediated by activation of the cell cycle inhibitor p21 (Cdkn1a) accompanied by telomere shortening. As mentioned elsewhere, p53, $p16^{Ink4A}$ and FOXO are key to trigger physiologic stress-induced stem cell aging thereby resulting in aged HSC phenotype.[55-58] These stressors are proven as surrogate for human aging and age-associated disorders.

Adult stem cells receive influential ageing cues from environmental changes, as young HSCs into aged microenvironment of telomerase wild-type mice also resulted in reduced function of HSCs. Interestingly, caloric restriction not only leads to life-span extension in mice, but also ameliorates HSC ageing.[59] Stem cells are typically characterized with enhanced levels of ABC multidrug transporter, Bcl-2 and Bcl-xL and surviving, which coherently defend the cytotoxic risk in the stem cell population.[60] Collectively, these studies further emphasize the intricate interplay between multiple extrinsic and intrinsic factors and pathways governing the aging of HSCs.

Stem cells and cancer

Accumulating evidence in recent years strongly indicates the existence of cancer stem cells in various solid tumors. A fascinating aspect is that cancer stem cells share many of the characteristics of normal stem cells, such as the ability to self-renew and to give rise to multiple types of differentiated cells.[61,62] In addition, some cancer stem cells might be direct descendants of normal tissue stem cells, indicating that normal stem cells can be the primary target of oncogenic transformation. Given that tumor initiation and progression require acquisition of multiple mutations during a long period, stem cells become a suitable target for the accumulation of mutations and eventually malignant transformation.[61] This has been largely attributed to the longevity of tissue stem cells, which makes stem cells prone to accrue more genomic alterations than their non-self-renewing progeny, leading to stem cell genetic instability, an engine for neoplastic transformation and malignancy development.[63] Although, any stem cell has a very rare probability of acquiring chromosomal aberrations or being subjected to cancer risk formation, the consequences could be profound, as only a very few cancer cells are sufficient to trigger tumorigenesis.[64] The cancer stem cells in the tumors not only initiate the tumor growth and sustain self-renewal, but also maintain metastatic potential thereby challenging the conventional cancer therapies more vigorously than the normal cancer cells.

Arguably, one of the most striking and direct con-

sequences of the genome instabilities is carcinogenesis. A hallmark of tumorigenesis is accumulation of genetic lesions in the cell cycle check points at a rate higher than in normal cells. Whereas the genome instabilities in tumors typically arise through states of continuous, sustaining, and perpetuating chromosomal mutations, irrespective of cytogenetic complexity or heterogeneity and genomic integrity per se. The cancer stem cell hypothesis has enormous implications for cancer therapeutics.[65] As current treatments targeting only rapidly proliferating cancer cells, a surviving small fraction of cancer stem cells might drive the secondary tumor recurrence in cancer patients. Thus, identification and targeting of the cancer stem cells are of great clinical importance.

Therapeutic implications of stem cells

It is apparent there are no simplistic chemotherapeutic approaches available for most debilitating disorders, such as degenerative disorders, cancers, and relevant tissue damage disorders. This roadblock in the treatment catalyzes enormous attention in the potential applications of stem cells. Due to their impressive self-renewal and differentiation potentials, adult stem cells hold great promise in cell and gene therapy applications for the treatment of many disorders.[66] Interestingly, tissue stem cells through their regenerative capability, are able to differentiate into residing tissue to partially restore the function. Mesenchymal stem cells from fetal bone marrow, for instance, are capable of differentiating into not only osteogenic, adipogenic and endothelial lineages, but also hepatocyte-like cells, chondrocytes, muscles, neural, and erythroid cells.[67] Interestingly, their regenerative and tissue repair potential are not restricted to their local milieu, but also to tissues of distal organs via pro-inflammatory cytokines and growth factors. The added benefit is that both autologous and allogenic stem cells have no immunoreactivity problems in systemic administration and local transplantation, rendering stem cells as an ideal choice to deliver the genes of interest in gene therapy applications in various tissues. Development of cell-specific gene therapeutic approaches are now underway to cure various diseases including premature aging diseases, diabetes, atherosclerosis, hematopoietic, cardiovascular, musculoskeletal, gastrointestinal, pulmonary, urogenital, ocular, neurodegenerative and skin disorders.[68]

A recent study shows that retroviral coexpression of telomerase RNA component (TER) and telomerase reverse transcriptase (TERT), could extend telomere length and rescue autosomal dominant form of dyskeratosis congenita cells from a phenotype characteristic of early senescence.[69] The majority of human cancer cells overexpress hTERT but their level is subdued in normal adult tissue[70] rendering them an attractive target for tumor therapy. hTERT immunotherapy involves eliciting cytotoxic T-lymphocyte (CTL)-mediated immune response in most cancers patients. Gene therapy comprising of oncolytic adenoviral vector carrying apoptotic TNF-related apoptosis-inducing ligand and E1A gene (Ad/TRAIL-E1) may preferentially target CSCs[71] and suppress tumor growth and survival. Similarly, many oncolytic adenoviral vector therapies have been aimed at treating breast, lung, prostate, pancreatic, and relevant cancers. Many potential therapeutic approaches were successfully developed by targeting genes involved in crucial stem cell pathways. For instance, Wnt, Notch, SCF, EGFR, Hedgehog, IL-4, Ras/MAPK, NF-kB and survivin signaling genes were targeted to eradicate resistance of cancer stem cells and also as adjuvants to conventional tumor therapeutics. Importantly, molecular targeting of BMI1 using DNA methylation inhibitor 5-azacytidine is able to control cancer stem cell growth.[72] Likewise, many Notch inhibitors, for instance,-secretase inhibitors were developed for cancer therapy in leukemic patients.[73] Anti-Wnt/β-catenin factors could destabilize the chronic lymphocytic leukemic cells[74] by forcing them into enhanced apoptosis. Similarly, therapy aimed at targeted overexpression of Hes1 is sufficient to protect Mbs and melanocyte stem cells (MSCs) from a severe defect in hair pigmentation, followed by intensive hair graying.[75] Many other therapeutic approaches, for instance, caloric restriction therapy supplemented with dietary natural compounds could maintain the longevity of the stem cells. Sirtuin-1, for instance, is required for calorie restriction-induced lifespan extension in mice, and calorie restriction upregulates sirtuin-1 in humans. Sirtuin-1 also appears to influence lineage/cell-fate decisions of stem cells via actively suppressing ROS levels to improve cell survival and cure type II diabetes mellitus like metabolic disorders.[59]

Conclusions and future directions

There has been tremendous progress in our understanding of stem cells in the past few years. Unraveling the molecular mechanisms of adult stem cells is a prerequisite to understand their causative roles in ageing, cancer and various degenerative disorders. Recent insights into the regulation of self-renewal and differentiation of stem cells raise several fundamental questions. Besides, underlying mechanisms that dictate stem cell behavior and their implications in ageing and cancer-like disorders have not been established. In addition, how the bone marrow niche regulate the fate of stem cells and

how stem cells respond to their signals remain to be answered. It is also intriguing to know how an organism drives the stem cell mobilization and their reestablishment at distal tissue organs in response to variety of stressors. The prevalence of complex degenerative disorders propagates our immense interest in development of novel therapeutic approaches against these disorders. High-throughput, multifaceted, and sophisticated approaches should be directed to gain more insights into the complex defense mechanisms of rare populations of cancer stem cells, which protect them against conventional therapies. In particular, by exploring seminal aspects of stem cells, the most-promising targeted therapies for various chronic degenerative disorders can be designed.

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REFERENCES

1. Cheshier SH, Morrison SJ, Liao X, Weissman IL. In vivo proliferation and cell cycle kinetics of long-term self-renewing hematopoietic stem cells. Proc Natl Acad Sci USA 1999;96:3120-125.
2. Passegue E, Wagers AJ, Giuriato S, Anderson WC, Weissman IL. Global analysis of proliferation and cell cycle gene expression in the regulation of hematopoietic stem and progenitor cell fates. J Exp Med 2005;202:1599-1611.
3. Zon LI. Intrinsic and extrinsic control of haematopoietic stem-cell self-renewal. Nature 2008;453:306-313.
4. Cellot S, Sauvageau G. Zfx: At the Crossroads of Survival and Self-Renewal 2007; 129(2):239-241.
5. He S, Nakada D, Morrison SJ. Mechanisms of stem cell self-renewal. Annu Rev Cell Dev Biol. 2009;25:16.1-16.30.
6. Molofsky AV, Pardal R, Morrison SJ. Diverse mechanisms regulate stem cell self-renewal. Curr Opin Cell Biol 2004;16:700-707.
7. Knoblich JA. Mechanisms of Asymmetric Stem Cell Division. Cell 2008;132: 583-597.
8. Rossi DJ, Bryder D, Seita J, Nussenzweig A, Hoeijmakers J, Weissman IL. Deficiencies in DNA damage repair limit the function of haematopoietic stem cells with age. Nature 2007;447:725-729.
9. Ivanova N, Dobrin R, Lu R, Kotenko I, Levorse J, DeCoste C, Schafer X, Lun Y, Lemischka IR. Dissecting self-renewal in stem cells with RNA interference. Nature 2006;442:533-538.
10. Dottori M, Gross MK, Labosky P, Goulding M. The winged-helix transcription factor Foxd3 suppresses interneuron differentiation and promotes neural crest cell fate. Development 2001;128:4127-4138.
11. Kos R, Reedy MV, Johnson RL, Erickson CA. The winged-helix transcription factor FoxD3 is important for establishing the neural crest lineage and repressing melanogenesis in avian embryos. Development 2001;128:1467-1479.
12. Xu D, Yoder M, Sutton J, Hromas R. Forced expression of Genesis, a winged helix transcriptional repressor isolated from embryonic stem cells, blocks granulocytic differentiation of 32D myeloid cells. Leukemia 1998;12:207-212.
13. Kirstetter P, Anderson K, Porse BT, Jacobsen SE, Nerlov C. Activation of the canonical Wnt pathway leads to loss of hematopoietic stem cell repopulation and multilineage differentiation block. Nature Immunol 2006;7:1048-1056.
14. Scheller M. Hematopoietic stem cell and multilineage defects generated by constitutive b-catenin activation. Nature Immunol. 2006;7:1037-1047.
15. Phillips RL, Ernst RE, Brunk B, Ivanova N, Mahan MA, Deanehan JK, Moore KA, Overton GC, Lemischka IR. The genetic program of hematopoietic stem cells. Science 2000;288:1635-1640.
16. Boiani, M. Eckardt S, Scholer HR, McLaughlin KJ. Oct4 distribution and level in mouse clones: consequences for pluripotency. Genes Dev 2002;16:1209-1219.
17. Chambers I, Colby D, Robertson M, Nichols J, Lee S, Tweedie S, Smith A. Functional expression cloning of Nanog, a pluripotency sustaining factor in embryonic stem cells. Cell 2003;113:643-655.
18. Galan-Caridad JM, Harel S, Arenzana TL, Hou ZE, Doetsch FK, Mirny LA, Reizis B. Zfx controls the self-renewal of embryonic and hematopoietic stem cells. Cell 2007;129(2):345-57.
19. Kozar K, Ciemerych MA, Rebel VI, Shigematsu H, Zagozdzon A. Mouse development and cell proliferation in the absence of D-cyclins. Cell 2004; 118:477-91.
20. Park IK, Qian D, Kiel M, Becker MW, Pihalja M, Weissman IL, Morrison SJ, Clarke MF. Bmi-1 is required for maintenance of adult self-renewing haematopoietic stem cells. Nature 2003;423:302-5.
21. Nerlov C, Graf T. PU.1 induces myeloid lineage commitment in multipotent hematopoietic progenitors. Genes Dev 1998;12:2403-2412.
22. Rekhtman N, Radparvar F, Evans T, Skoultchi AI. Direct interaction of hematopoietic transcription factors PU.1 and GATA-1: functional antagonism in erythroid cells. Genes Dev 1999;13:1398-1411.
23. Galloway JL, Wingert RA, Thisse C, Thisse B, Zon LI. Loss of gata1 but not gata2 converts erythropoiesis to myelopoiesis in zebrafish embryos. Dev Cell 2005;8:109-116.
24. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006;126:663-676.
25. Oguro H, Iwama A, Morita Y, Kamijo T, van Lohuizen M, Nakauchi H. Differential impact of Ink4[a] and Arf on hematopoietic stem cells and their bone marrow microenvironment in Bmi1-deficient mice. J Exp Med 2006;203:2247-2253.
26. Krivtsov AV, Twomey D, Feng Z, Stubbs MC, Wang Y, Faber J, Levine JE, Wang J, Hahn WC, Gilliland DG, Golub TR, Armstrong SA. Transformation from committed progenitor to leukaemia stem cell initiated by MLL-AF9. Nature 2006; 442:818-822.
27. Ernst P, Fisher JK, Avery W, Wade S, Foy D, Korsmeyer SJ. Definitive hematopoiesis requires the mixed-lineage leukemia gene. Dev Cell 2004;6:437-443.
28. Arai F, Hirao A, Ohmura M, Sato H, Matsuoka S. Tie2/angiopoietin-1 signaling regulates hematopoietic stem cell quiescence in the bone marrow niche. Cell 2004;118:149-61.
29. Kollet O, Dar A, Shivtiel S, Kalinkovich A, Lapid K. Osteoclasts degrade endosteal components and promote mobilization of hematopoietic progenitor cells. Nat Med 12:657-64.
30. Wilson A, Trumpp A. Bone-marrow haematopoietic-stem-cell niches. Nature Reviews Immunology 2006;6:93-106.
31. Shiotsugu J, Katsuyama Y, Arima K, Baxter A, Koide T, Song J, Chandraratna RA, Blumberg B. Multiple points of interaction between retinoic acid and FGF signaling during embryonic axis formation. Development 2004;131:2653-2567.
32. Nordstrom U, Maier E, Jessell TM, Edlund T. An early role for WNT signaling in specifying neural patterns of Cdx and Hox gene expression and motor neuron subtype identity. PLoS Biol 2006;4: e252.
33. North TE, Goessling W, Walkley CR, Lengerke C, Kopani KR, Lord AM, Weber GJ, Bowman TV, Jang IH, Grosser T, Fitzgerald GA, Daley GQ, Orkin SH, Zon LI. Prostaglandin E2 regulates vertebrate haematopoietic stem cell homeostasis. Nature 2007;447: 1007-1011.
34. Karpowicz P, Morshead C, Kam A, Jervis E, Ramunas J, Cheng V, van der Kooy D. Support for the immortal strand hypothesis: neural stem cells partition DNA asymmetrically in vitro. J Cell Biol 2005;170:721-732.
35. Potten CS, Owen G, Booth D. Intestinal stem cells protect their genome by selective segregation of template DNA strands. J Cell Sci 2002;115:2381-2388.
36. Nijnik A, Woodbine L, Marchetti C, Dawson S, Lambe, T, Liu C, Rodrigues NP, Crockford TL, Cabuy E, Vindigni A, Enver T, Bell JI, Slijepcevic P, Goodnow CC, Jeggo PA, Cornall RJ. DNA repair is limiting for haematopoietic stem cells during ageing. Nature 2007;447:686-690.
37. Taussig DC, Oearce DJ, Simpson C, Rohatiner AZ, Lister TA, Kelly G, Luongo JL, Danet-Desnoyers GH, Bonnet D. Hematopoietic stem cells express multiple myeloid markers: implications for the origin and targeted therapy of acute myeloid leukemia. Blood 2005;106:4086-4092.
38. Holyoake T, Jiang X, Eaves C, Eaves A. Isolation of a highly quiescent subpopulation of primitive leukemic cells in chronic myeloid leukemia. Blood 1999;94:2056-2064.
39. Graham SM, Jorgensen HG, Allan E, Pearson C, Alcorn MJ, Richmond L, Holyoake TL. Primitive, quiescent, Philadelphia-positive stem cells from patients with chronic myeloid leukemia is insensitive to STI571 in vitro. Blood 2002;99:319-325.
40. Akashi K, Traver D, Miyamoto T, Weissman IL. A clonogenic common myeloid progenitor that gives rise to all myeloid lineage. Nature 2000; 404:193-197.

41. Rossi DJ, Seita J, Czechowicz A, Bhattacharya D, Bryder D, Weissman IL. Hematopoietic stem cell quiescence attenuates DNA damage response and permits DNA damage accumulation during aging. Cell Cycle 2007; 6:2371-2376.
42. Park Y, Gerson SL. DNA repair defects in stem cell function and aging. Annu Rev Med. 2005;56:495-508.
43. Ruzankina Y, Asare A, Brown EJ. Replicative stress, stem cells and aging. Mech Ageing Dev 2008;129:460-466.
44. Sharpless NE, DePinho RA. How stem cells age and why this makes us grow old. Nat Rev Mol Cell Biol 2007;8:703-713.
45. Rando TA. Stem cells, ageing and the quest for immortality. Nature 2006;441:1080-1086.
46. Carlson ME, hsu M, Conboy IM. Imbalance between pSmad3 and Notch induces CDK inhibitors in old muscle stem cells. Nature 2008;454:528-532.
47. Hiyama E, Hiyama K. Telomere and telomerase in stem cells. Br J Cancer 2007;96:1020-1024.
48. Arai F, Suda T. Maintenance of quiescent hematopoietic stem cells in the osteoblastic niche. Ann NY Acad Sci 2007;1106:41-53.
49. Aubert G, Lansdorp PM. Telomeres and aging. Physiol Rev 2008;88:557-579.
50. Choudhury AR, Ju Z, Djojosubroto MW, Schienke A, Lechel A, Schaetzlein S. p53 deficiency rescues the adverse effects of telomere loss and cooperates with telomere dysfunction to accelerate carcinogenesis. Cell 1999; 57:527–538.
51. Armstrong L, Saretzki G, Peters H, Wappler I, Evans J, Hole N, von Zglinicki T, Lako M. Overexpression of telomerase confers growth advantage, stress resistance, and enhanced differentiation of ESCs toward the hematopoietic lineage. Stem Cells 2005;23:516–529.
52. Choudhury AR, Ju Z, Djojosubroto MW, Schienke A, Lechel A, Schaetzlein S, Jiang H, Stepczynska A, Wang C, Buer J, Lee HW, von Zglinicki T, Ganser A, Schirmacher P, Nakauchi H, Rudolph KL. Cdkn1^{a} deletion improves stem cell function and lifespan of mice with dysfunctional telomeres without accelerating cancer formation. Nature Genet 2007;39: 99-105.
53. Ju Z, Jiang H, Jaworski M, Rathinam C, Gompf A, Klein C, Trumpp A, Rudolph KL. Telomere dysfunction induces environmental alterations limiting hematopoietic stem cell function and engraftment. Nat Med 2007;13(6):742-747.
54. Rudolph KL, Chang S, Lee HW, Blasco M, Gottlieb GJ, Greider C, DePinho RA. Longevity, stress response, and cancer in aging telomerase-deficient mice. Cell 1999;96:701-712.
55. Janzen V, Forkert R, Fleming H, Saito Y, Waring M, Dombkowski D, Cheng T, DePinho R, Sharpless N, Scadden D. Stem-cell ageing modified by the cyclin-dependent kinase inhibitor p16(INK4^{a}). Nature 2006;443:421-426.
56. Dumble M, Moore L, Chambers SM, Geiger H, Van Zant G, Goodell MA, Donehower LA. The impact of altered p53 dosage on hematopoietic stem cell dynamics during aging. Blood 2007;109:1736-1742.
57. Gatza C, Moore L, Dumble M, Donehower LA. Tumor suppressor dosage regulates stem cell dynamics during aging. Cell Cycle 2007;6:52-55.
58. Tothova Z, Gilliland DG. FoxO transcription factors and stem cell homeostasis: insights from the hematopoietic system. Cell Stem Cell 2007;1:140-152.
59. Mantel C, Broxmeyer HE. Sirtuin 1, stem cells, aging, and stem cell aging. Curr Opin Hematol. 2008;15:326-331.
60. Krishnamurthy P, Schuetz JD. Role of ABCG2/BCRP in biology and medicine. Annu Rev Pharmacol Toxicol 2006;46:381-410.
61. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells, Nature 2001;414:105-111.
62. Pardal R, Clarke MF, Morrison SJ. Applying the principles of stem-cell biology to cancer. Nat Rev Cancer 2003;3(12):895-902.
63. Pazhanisamy S, Jyothi V. Genome architecture, instabilities, stem cells and cancer. J Stem Cell & Regen Med. 2009;4(1).
64. Kreth G, Pazhanisamy S, Hausmann M, Cremer C. Cell Type-Specific Quantitative Predictions of Radiation-Induced Chromosome Aberrations: A Computer Model Approach. Radiation Research May 2007;167(5):515-525.
65. Jordan CT, Guzman ML, Noble M. Cancer stem cells. N Engl J Med 2006;355(12):1253-61.
66. Pazhanisamy S. Stem Cell Research: The current progress and prospective applications. Drug Lines. 2002;4(1 & 2):1-5.
67. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD. Multilineage potential of adult human mesenchymal stem cells, Science 1999; 284:143-147.
68. Mimeault M, Batra SK. Recent insights into the molecular mechanisms involved in aging and the malignant transformation of adult stem/progenitor cells and their therapeutic implications. Ageing Res Rev 2009;8(2):94-112.
69. Westin ER, Chavez E, Lee KM, Gourronc FA, Riley S, Lansdorp PM, Goldman FD, Klingelhutz AJ. Telomere restoration and extension of proliferative lifespan in dyskeratosis congenita fibroblasts. Aging Cell 2007;6(3):383-94.
70. Carpenter EL, Vonderheide RH. Telomerase-based immunotherapy of cancer. Expert Op on Biol Therapy 2006;6(10):1031-1039.
71. Zhang X, Komakl R, Wang L, Fang B, Chang JY. Treatment of Radioresistant Stem-Like Esophageal Cancer Cells by an Apoptotic Gene-Armed, Telomerase-Specific Oncolytic Adenovirus. Clin Cancer Res 2008;14:2813.
72. Hernandez-Munoz I, Taghavi P, Kuijl C, Neefjes J, van Lohuizen M. Association of BMI1 with polycomb bodies is dynamic and requires PRC2/EZH2 and the maintenance DNA methyltransferase DNMT1. Mol Cell Biol 2005;25:11047-58.
73. Deangelo DJ, Stone RM, Silverman LB. A phase I clinical trial of the notch inhibitor MK-0752 in patients with T-cell acute lymphoblastic leukemia/lymphoma (T-ALL) and other leukemias. J Clin Oncol 2006;24:6585.
74. Lu D, Zhao Y, Tawatao R. Activation of the Wnt signaling pathway in chronic lymphocytic leukemia. Proc Natl Acad Sci USA 2004;101:3118-23.
75. Moriyama M, Osawa M, Mak SS, Ohtsuka T, Yamamoto N, Han H, Delmas V, Kageyama R, Beermann F, Larue L, Nishikawa S. Notch signaling via Hes1 transcription factor maintains survival of melanoblasts and melanocyte stem cells. J Cell Biol. 2006;173:333-339.