Genome integrity, stem cells and hyaluronan

Zbigniew Darzynkiewicz and Endre A. Balazs

1 Brander Cancer Research Institute & Department of Pathology, New York Medical College, Valhalla, NY 10595, USA
2 Matrix Biology Institute, 725 River Road, Suite 205, Edgewater, NJ 07020, USA

Key words: Hyaluronic acid, stem cells, stem cells niche, germinal cells, sperm cells, oocytes, oxidative DNA damage, reactive oxidative species (ROS); inflammation, cell cycle, apoptosis

Received: 2/19/12; Accepted: 2/24/12; Published: 2/26/12

Correspondence to: Zbigniew Darzynkiewicz, MD/PhD; E-mail: darzynk@nymc.edu

Abstract: Faithful preservation of genome integrity is the critical mission of stem cells as well as of germ cells. Reviewed are the following mechanisms involved in protecting DNA in these cells: (a) The efflux machinery that can pump out variety of genotoxins in ATP-dependent manner; (b) the mechanisms maintaining minimal metabolic activity which reduces generation of reactive oxidants, by-products of aerobic respiration; (c) the role of hypoxic niche of stem cells providing a gradient of variable oxygen tension; (d) the presence of hyaluronan (HA) and HA receptors on stem cells and in the niche; (f) the role of HA in protecting DNA from oxidative damage; (g) the specific function of HA in protecting DNA in stem cells; (h) the interactions of HA with sperm cells and oocytes that also may shield their DNA from oxidative damage, and (e) mechanisms by which HA exerts the anti-oxidant activity. While HA has multitude of functions its anti-oxidant capabilities are often overlooked but may be of significance in preservation of integrity of stem and germ cells genome.

Stem cells: keeping genotoxins out of the cell

Faithful preservation of genome integrity throughout lifetime of the organism is the critical mission of the long-term self-renewal stem cells. Several mechanisms, both intrinsic to stem cells themselves as well as extrinsic, provided by the microenvironment, (stem cell niche) serve this purpose. One of the intrinsic mechanisms is aimed to effectively exclude potentially hazardous agents that enter the cell from outside. This activity is mediated by high level of expression of multidrug-resistance gene (MDR1)-encoded adenosine triphosphate-binding cassette (ABC) transporter P-glycoprotein (P-gp). This efflux machinery can pump out variety of genotoxins in ATP-dependent manner [1]. There are over 30 members of ABC transporter superfamily genes, whose protein transcripts are able to remove wide range of substrates out of the cell [2-4]. The degree of efflux activity correlates with differentiation; namely the maximal activity are expressing the most primitive long-term self-renewal stem cells [5]. Another possible function for these efflux pumps is the removal of small lipophilic regulatory molecules such as steroids, whose presence may activate growth or differentiation [6].

It should be noted that the exceptionally high activity of the efflux pump provides a useful marker to identify and isolate (sort out) stem and early progenitor cells. This is being achieved using fluorescent efflux substrates such as Hoechst 33342 [5,7,8] or rhodamine 123 [9,10] in conjunction with flow cytometry and electronic cell sorting. Since retention of these fluorochromes in stem cells is impeded due to their rapid efflux, attributed primarily to MDR1 activity [11], stem cells can be recognized (and sorted out) as a distinct cell subpopulation characterized by much reduced fluorescence intensity. In the case of staining with Hoechst 33342 the hematopoietic stem cells are characterized by the reduced intensity of fluorescence combined with a metachromatic shift of this fluorochrome, revealed as so called “side population”,...
having much distinct fluorescence emission properties compared to other bone marrow cells [5,8].

**Oxygen danger**

One of the strong genotoxins is oxygen [12,13]. Oxidative DNA damage leads to oxidation of DNA bases with predominance of guanine [formation of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxo-dG)], base ring fragmentation, sugar modification, crosslinking of DNA and protein as well as induction of DNA strand breaks [14,15]. The most deleterious effect of oxidative DNA damage is formation of DNA double-strand breaks (DSBs). These lesions can be repaired either by recombinatorial repair or non-homologous DNA-end joining (NHEJ). The template-assisted recombinatorial repair is essentially error-free but can take place when cells have already replicated their DNA which serves as a template, i.e. in late-S and G2 phase of the cell cycle. DNA repair in cells lacking a template (G1 and early S phase) occurs by the NHEJ mechanism. The latter however, is error-prone and may result in deletion of some base pairs [16]. When such change is at the site of an oncogene or tumor suppressor gene it may promote tumorigenesis [17]. It can also cause translocations and telomere fusion [18]. When the stem cells reside in the in the non-proliferating G0 state the error-prone NHEJ is the only mechanism for their DSBs repair.

Both, the exogenous oxygen as well as reactive oxygen species (ROS), the by-products of aerobic respiration within the cell, contribute to oxidative DNA damage [19-20]. The primary ROS generated in mitochondria can diffuse from these organelles and reach the nuclear DNA inducing its damage, or can generate secondary radicals with DNA damaging properties [21,22]. The ongoing oxidative DNA damage by endogenous oxidants induces consistent replication stress, which when concurrent with activation of mTOR pathways, is considered to be the primary cause of cell senescence and organismal aging [23-28]. Clearly, the genome of stem cells has to be maximally protected from senescing- and aging-related changes. What mechanisms operate to shield genomic DNA in stem cells from the oxidative damage?

**Reduced oxygen level in the environment: the hypoxic niche**

One of the factors reducing oxidative DNA damage in stem cells is their location. Stem cells reside in their respective niches [29], the distinct anatomical compartments composed of cellular and intercellular matrix constituents providing an optimal milieu for maintenance and regulation of their biologic processes [30]. The very characteristic feature of stem cell niche is low concentration of oxygen [31,32]. While percentage of oxygen in ambient air is about 21% [partial pressure, oxygen tension p(O2) = 180 mm Hg], its percentage in most organs and tissues is reduced to 2% - 9% (15 - 68 mm Hg) [33,34] and in the environment of stem cell niche is between 1% and 6% (7.6 – 45 mm Hg) and the intracellular p(O2) is 3 – 4 mm Hg [35,36].

Interestingly, at the very low oxygen tension (1%) the stem cells remain in the non-proliferating compartment and maintain pluripotency while at the increased tension (3% - 5%) they proliferate and start differentiation [36,37]. The oxygen tension gradient within the stem cell niche appears to provide signaling that mediates transition of these cells from quiescence to proliferation and trigger their differentiation [38-42]. At the same time the very low oxygen tension milieu, where the long-term self-renewal stem cells reside, offers the conditions in which oxidative DNA damage induced by exogenous oxygen is much reduced. Based on differences in p(O2) such damage is expected to be nearly 20-fold lower in the stem cell niche than in tissue culture maintained at ambient air.

**Containing danger of endogenous oxidants**

Several mechanisms are being used by stem cells to mitigate the hazards conveyed by the endogenous reactive oxidants. First of all, for most of their life stem cells remain in quiescent state having minimal metabolic rate and thereby low level of ROS production [43-47]. Their quiescent state is comparable to that of the peripheral blood lymphocytes, whose metabolic rate and generation of oxidants is nearly two orders of magnitude lower that of their mitogenically stimulated counterparts [48,49]. The metabolic rate of the most primitive stem cells, the “very small embryonic-epiblast stem cells”(VSELs), as judged from extreme paucity of their cytoplasmic content and few mitochondria, is expected to be indeed minimal [50].

Further mechanism designed to maintain minimal metabolic rate may involve asymmetric cell division. It was recently shown that the most primitive, long-term reconstituting, hematopoietic stem cells (Lin- Sca1+Kit+; LSK) which continuously reside in the LSK compartment, during division segregate their mitochondria unequally delegating more of these organelles to that of the daughter cell which exits the LSK compartment and enters differentiation pathway. The sister cell remaining in the LSK compartment inherits the very low level of energized mitochondria and thus remains with minimal level of ROS production [51,52].
Hyaluronan in stem cells biology

Hyaluronan (hyaluronic acid; HA), the ubiquitous component of intercellular matrix, carries out numerous functions that are essential for survival, differentiation, proliferation, motility and intercellular communication of variety of cell types. HA is also of crucial importance for tissue and organ development and architecture [reviews in 53]. Depending on its molecular weight HA may exert diverse effects on normal and cancer cells functions [53-57]. Extensive evidence points out that HA plays critical role in many facets of stem cells biology. Most of the evidence comes from the studies of Susan Nilsson and her collaborators of the Peter MacCallum Cancer Center, Melbourne, who explored the interactions of this glycosaminoglycan with human and murine hematopoietic stem cells [55-59]. These authors demonstrated that HA is being synthesized by HA-synthases located in the plasma membrane of stem cells and is expressed on the surface of these cells [44,56]. Three HA synthases, HAS-1, HAS-2 and HAS-3, each synthesizing this biopolymer at different molecular weight, are involved in its production in stem cells. HA is also a predominant component of intercellular matrix of the stem cell niche. It is unclear to what extent stem cells themselves and the stromal cells (fibroblasts) respectively contribute to its accumulation in the niche [59,60].

The level of expression of HA on the cells surface correlates with the differentiation status of the stem and progenitor cells. The highest HA expression is observed on the surface of the most primitive (Lin-) stem cells, and a progressive decline in its expression is seen to be concurrent with differentiation. The mechanistic in vitro studies indicate that HA functions in modulating cell proclivity to differentiation and proliferation by enforcing continuance of the dormant state of the most primitive Lin- cells [55-61]. This inhibitory effect of HA is consistent with its suppressive effect on mitogenic stimulation of lymphocytes which also remain in the G0 dormant state when cultured with the mitogen in the presence of high molecular weight HA [62].

HA plays also essential role in homing and lodgment of the transplanted stem cells. The transplanted hematopoietic stem and progenitor cells preferentially home to the trabecule-rich metaphysic of femur where they become lodged in endosteal niche, being associated with blood vessels. The presence of HA, which is highly expressed in endothelium of blood vessels, provides the homing and lodgment mechanism for the stem cells having strong expression of the HA receptors CD44 and RHAMM [57,63,64]. HA-synthase HAS-3 plays a major role in this mechanisms because transplantation of stem cells to mice lacking this synthase (Has-3−/−) leads to aberrant distribution of the grafted cells in bone marrow compared to the wild type recipients [58]. Apparently the presence of active HAS-1 and HAS-2 synthases is inadequate to assure proper homing and lodging of hematopoietic stem cells in Has-3−/− mice. The presence of HA CD44 receptors on surface of stem cells mediates their adhesion and rolling movement on HA surfaces [65].

One of the mechanisms by which HA facilitates homing and engraftment of transplanted hematopoietic stem cells (as shown in the case of umbilical cord blood) involves its effect in promoting synthesis of membrane type 1 (MT1) of matrix metalloproteinase 2 (MMP-2) [66]. Since MT1-MMP-2 plays an important role in homing of hematopoietic progenitor stem cells it has been proposed that the priming strategy that involves pretreatment of cord blood progenitor/stem cells with HA before transplantation could improve their homing and engraftment [66].

Interestingly, HA can substitute hypoxia for the long-term maintenance of embryonic stem cells in culture, preserving their pluripotency and thereby providing a useful alternative that enhances their viability [67]. A combination of hypoxia and HA may be even more beneficial in this application.

The importance and protective effect of HA on stem cells are well recognized in the field of transplantation of these cells in regenerative medicine. Variety of hydrogel scaffolds providing a niche-like environment, being used as stem cell vehicles for their transplantation [68-73]. HA, modified by different approaches to allow structural encapsulation of individual stem cells, is the key component of all these hydrogel products. Stem or progenitor cells encapsulated into such bio-artificial niches have been found to be protected from cytotoxic agents such as anticancer drugs used to treat the patient, and remain competent in terms of their capability to lodge and functionally replace native stem cells [69-73].

It should be noted that the HA receptor CD44 is frequently used as a marker to identify and sort out drug-resistant stem-like cells from different tumors [74-76]. Interactions between HA and cancer cells are the subject of extensive literature [reviews in 77] but little is known on the role of HA and HA-CD44 interactions in cancer stem-like cells, particularly whether such interactions may affect stability of these cells genome. However, there are interesting observations from the studies of cancer cells pertaining to interactions between CD44 and...
HA vis-à-vis the efflux pump in these cells affecting efficiency of the P-glycoprotein in removing anticancer drugs of known genotoxicity [78-81]. It is possible that the HA-CD44 interactions in cell membrane of stem cells play a role in protecting their genome via modulation of the efflux pump effectiveness.

**Protection of DNA from oxidative damage by hyaluronan**

The increased production of ROS takes place during inflammation and the oxidants generated in the inflamed tissue mediate and further amplify many inflammatory reactions [82-87]. It has been proposed that one of HA biological functions is to provide protection against cellular damage caused by radicals generated by oxidative reductive systems or ionizing radiation [88]. Indeed, HA has strong anti-inflammatory properties and is clinically used to abrogate or attenuate inflammation in many tissues and organs [89-100]. Its clinical utility is of particular significance in treatment of osteoarthritis [89,95-97,100]. The anti-inflammatory capabilities of HA are mediated, at least in large part, by its antioxidant activity [101-108]. Promotion of the wound healing by HA may also be facilitated by similar antioxidant mechanisms [109,110].

We have recently reported that DNA damage signaling evoked by exposure of cells to exogenous oxidants such as H₂O₂ was markedly attenuated by HA; especially effective was HA of high molecular weight [111]. In another study, we observed that the extent of DNA damage response induced during oxidative burst of macrophages [112] was also distinctly reduced in the presence of high molecular weight HA [113]. Moreover, the level of constitutive DNA damage signaling revealed by activation of Ataxia Telangiectasia Mutated (ATM) and phosphorylation of histone H2AX on Ser139 and reporting persistent DNA damage by endogenous oxidants, the by-products of aerobic respiration in mitochondria [114,115], was seen to be strongly attenuated by HA [111]. Here again, the high molecular weight HA was more effective than its low molecular weight form [113]. These findings underscore that HA exerts a protective effect on cellular genome by neutralizing the exogenous as well as endogenous oxidants.

Protection of DNA by HA through its antioxidant activity has been also observed in fibroblast cultures in which oxidative stress was induced by treatment with iron and ascorbate [116]. In addition to protection of DNA integrity (assessed by analysis of its fragmentation) HA also reduced protein oxidation, lipid peroxidation and formation of OH* radicals in these cultures [116].

**Does hyaluronan also protect germ cells DNA?**

Interestingly, HA has found wide utility in assessment of integrity of sperm cells DNA. Specifically one of the methods to separate fertile sperm cells that have undamaged DNA is based on the ability of sperm cells to attach to HA [117-121]. The sperm cells that bind to solid-state HA show chromatin structure with high DNA chain integrity [116-120]. DNA integrity of HA-bound sperm cells was comparable to that assessed by the alternative methods of spermatozoa evaluation, based on DNA susceptibility to denaturation [122] or analysis of the presence of DNA strand breaks by the TUNEL assay [123]. Sperm cells express HA-receptors on the cell surface and it has been postulated that the HA-binding receptors have a specific role in cell maturation, motility and fertilization processes [124-126]. Since the cells expressing HA-receptors can internalize HA by endocytosis [127-129] it is possible that HA is internalized into spermatozoa and its presence within the cell can provide further DNA protection from oxidative damage. Since in sperm cells the ROS-generating organelles mitochondria are at some distance from nuclear DNA the HA-protection from exogenous rather than endogenous oxidants may be of more significance.

Oocytes were shown also to synthesize and secrete HA [130-133]. The concentration of HA in follicular fluid was shown to a good indicator for estimation of oocyte viability for fertilization [130]. The interactions between HA and CD44 receptors on cumulus-oocyte complexes were shown to be critical for oocyte maturation [133,134].

Similar to stem cells there are several mechanisms protecting DNA in germ cells, including the blood-testis barrier and efflux pumps [135-137]. The possible role of HA in terms of protection of germinal cells DNA against oxidative damage has not been addressed in the literature as yet. However, the collective evidence of strong correlation between the expression of HA-receptors versus genome integrity of sperm cells [119-122] as well as versus viability and capability of oocytes for fertilization [132], combined with the findings that HA protects DNA from oxidative damage in other cell types [111-113,116], provides a contention that HA may play a role in protecting germ cells genome from oxidative damage.

**Mechanisms of cell protection from oxidative damage by HA**

As discussed, strong evidence points out that the antioxidant properties of HA provide protection of
genomic DNA against the damage by ROS as well as are involved in attenuation of inflammatory processes. Two different mechanisms may contribute to the antioxidant properties of HA. One mechanism involves the ability of HA to chelate Fe++ and Cu++ [138,139]. These ions are critical in Fenton's reaction, in which the superoxide and hydroperoxide, which themselves are not highly reactive with DNA, are converted into the strongly reactive with DNA hydroxyl radical (OH*) [140-142]. Interception of these ions by HA makes them unavailable for Fenton’s reaction thereby reducing generation of the OH* radical.

The second mechanism by which HA may attenuate damage induced by oxidants involves the direct scavenging of oxidant molecules, particularly the reactive products of Fenton's reaction OH* radicals, by HA [101,103,116]. Of note, HA from synovial fluid was shown to have greater ROS-removing activity and scavenged more diverse range of ROS compared to other antioxidants such as catalase or superoxide dismutase (SOD) [103].

The ROS scavenging by HA while it depletes the pool of reactive oxidants potentially damaging DNA at the same time results in a breakdown of HA molecules [101,103,104]. The loss of HA viscosity, reporting HA degradation, is observed in vitro upon HA exposure to free radicals and in vivo, in the inflamed tissues [143]. It was shown that exposure of synovial fibroblasts to H2O2 while leads to HA breakage it also enhances HA synthesis [144]. Likely, this is a compensatory mechanism to maintain high level of HA thereby enhancing the antioxidant defense [145]. Because the newly synthesized HA is of high molecular weight and is replacing the degraded HA the capability of ROS scavenging is being preserved and not decreased.

When DNA damage occurs the highly effective and as much as possible error-free DNA repair is the subsequent mechanism by which stem cells protect genome integrity. Robustness of DNA repair in stem cells, and in particular of embryonic stem cells, is greater compared to other somatic cells [145-148]. For example following DNA damage mouse embryonic stem cells end up with 100 times fewer cells with mutations compared to mouse adult somatic or embryonic fibroblasts [145]. One mechanisms contributing to such an outcome is modulation of the cell cycle checkpoints and DNA damage signaling pathways to enhance efficiency of DNA repair. The second mechanism relies on elimination of cells with mutations by apoptosis. The checkpoint and DNA damage signaling pathways as well as the pathways regulating cell proclivity to undergo apoptosis are much more effective in stem cells compared to other somatic cells [145].

Possible pitfalls in interpretation of the data from in vitro experiments

Most observations regarding modulation of cell growth and proliferation of stem cells by HA came from the in vitro experiments in which the cells were growing at standard tissue culture conditions in an atmosphere of air and CO2. As mentioned, the partial pressure of O2 (pO2) of 159 mm Hg (21%) in ambient air is much higher than in tissues, especially in the microenvironment of the stem cells niche [35,36]. Because cell growth, and in particular the induction of proliferative senescence [149] as well as accumulation of mutations [150], are much dependant on oxygen concentration, the in vitro findings on cells growing under standard atmosphere conditions may be hampered by an experimental bias, i.e., the consequence of exposure to non-physiological pO2.

Most of the in vitro studies were also carried on stem cells growing attached to glass or plastic surfaces and exposed to HA that was included in the culture media. Such cells when attached to solid, dried out surfaces treated with various chemicals have very different migration properties, proliferation capability, and cell-to-cell interactions compared to in vivo conditions. The cells growing on solid surfaces exhibit also a very different behavior than that of the same cells grown on highly viscoelastic HA.

When cells from the lymphomyeloid system, tumor cells, fibrocytes or stem cells are placed on highly hydrated elastoviscous solutions of HA, they remain round, divide and move toward each other and on top of each other, forming a multilayered assembly. These assemblies are separated from each other. Depending on the cell lines, they survive or die slowly [53, 62, 103, 151,152]. This is the same highly purified hyaluronan that today is used worldwide as a therapeutic device in eye surgery, replacing the synovial fluid to control pain and as tissue augmentation of connective tissues of the skin and muscles.

Since in the vertebrate intercellular matrix, especially in the connective tissues, the cells are surrounded by liquid hyaluronan of various molecular weights and concentrations, it is logical to argue that the cells must have different behavior and activities – even gene expression – when surrounded with hyaluronan and not growing on plastic or glass surfaces. A caution, therefore, should be exercised in interpolating the in vitro results of cells growing on solid surfaces with the
in vivo conditions when the cells are embedded and exposed in a highly hydrated intercellular matrix. The cell-to-cell interactions may have an entirely different character.

Conclusions

The mechanisms associated with maintenance of genome integrity of stem cells, particularly focused on elucidation of the role of ROS and DNA damage response, have been recently reviewed [153]. In this review, while we briefly outline most mechanisms, the emphasis is given to the role of HA, which was neglected in prior literature. Although most reviewed data comes from hematopoietic stem cells attempts have been made to summarize the evidence on a role of HA in other types of stem cells as well. Protection of genome of the germ cells (spermatozoa and oocytes) is even of more importance that of the stem cells. Since there is a significant association between HA and expression of HA receptors and genome integrity in spermatozoa and oocytes we draw therefore attention to the possible role of HA in protection of DNA integrity in germ cells as well, which was also uncared for in prior reviews.

As yet there is no direct experimental evidence that unequivocally demonstrates that HA protects stem cells from oxidative DNA damage. However, the wealth of indirect evidence presented in this review strongly advances the notion that one of the functions of HA associated with stem cells is DNA protection against oxidative damage. The most supporting evidence to this conception provide the findings that DNA damage response triggered by exogenous as well endogenous oxidants in several cell types, most likely reporting persistent oxidative damage, is strongly attenuated by HA [111,113]. There is no rationale to expect that stem cells would be an exception and that DNA protection by this mechanism is not attainable in them. Since stem cells reside in the HA-rich niche, synthesizes HA, express it on the surface and are able to internalize it [55-59], clearly conditions exist that allow HA to provide protection of DNA against oxidants.

Of interest and of further support for this notion are observations that the level of expression of HA on surface of stem cells correlates with their differentiation status and the mechanistic studies showing that HA modulates cell proclivity to differentiation and proliferation [55-61]. On the other hand, there are observations that oxygen tension gradient within the stem cell niche appears to provide signaling that mediates transition of these cells from quiescence to proliferation and trigger their differentiation [38-42,154-156]. It is tempting therefore to speculate that the oxygen tension gradient which mediates propensity of stem cells to differentiate is actually being modulated by the gradient of HA associated with stem cells. Thus, HA could have diverse functions with respect to stem cells. Specifically it can: (i) protect the most primitive long-term self-renewal stem cells from reactive oxidants; (ii) offer a gradient of accessibility of ROS which trigger their differentiation. This is achieved by different expression of HA-receptors and thus different level of HA binding and possibly its internalization, (iii) modulate motility of stem cells and progenitors [65,70,157], and (iv) similar to another glycosaminoglycan, heparin sulfate [158,159], it may modulate gradients and accessibility to growth factors, and thus to control autocrine and paracrine signals for stem cells [158].

ACKNOWLEDGEMENTS

We thank Dr. Paul Lukas for reading draft of the manuscript and for his helpful comments. Supported by NCI RO1 CA 28704 and Matrix Biology Institute.

CONFLICT OF INTERESTS STATEMENT

The authors of this manuscript have no conflict of interest to declare.

REFERENCES

1. Scharenberg C, Harkey MA, Torok-Sorb B. The ABCG2 transporter is an efficient Hoechst 33342 efflux pump and is preferentially expressed in immature hematopoietic progenitors. Blood 2001;99:507-512.
2. Sissung TM, Baum CE, Kirkland CT, Geo R, Gardner ER, Figg WD. Pharmacogenetics of membrane transporters: an update on current approaches. Mol Biotechnol 2010;44:152-167.
3. Dean M, Allikmets R. Evolution of ATP-binding cassette transporter genes. Curr Opin Genet Dev. 1995;5:779-785.
4. Klein I, Sarkadi B, Varadi A. An inventory of the human ABC proteins. Biochim Biophys Acta. 1999;1461:237-262.
5. Goodell MA, Rosenzweig M, Kim H, Marks DF, DeMaria M, Paradis G, Grupp SA, Sief CA, Mulligan RC, Johnson RP. Dye efflux studies suggest that hematopoietic stem cells expressing low or undetectable levels of CD34 antigen exist in multiple species. Nat Med 1997;3:1337-1345.
6. Bunting KD, Galipeau J, Topham D, Benaim E, Sorrentino BP. Transduction of murine bone marrow cells with an MDR1 vector enables ex vivo stem cell expansion, but these expanded grafts cause a myeloproliferative syndrome in transplanted mice. Blood 1998;92:2269-2279.
7. Goodell MA, Brose K, Paradis G, Conner AS, Mulligan RC. Isolation and functional properties of murine hematopoietic stem cells that are replicating in vivo. J Exp Med 1996;183:1797-1806.
8. Lin KK, Goodell MA. Detection of hematopoietic stem cells by flow cytometry. Methods Cell Biol 2011;103:2130.
9. Darzynkiewicz Z, Staiano-Coico L, Melamed MR. Increased mitochondrial uptake of rhodamine 123 during lymphocyte stimulation. Proc Natl Acad Sci USA 1981; 78:2383-2387.
10. McKenzie JL, Takenaka K, Gan Ol, Doedens M, Dick JE. Low rhodamine 123 retention identifies long-term human hematopoietic stem cells within the LinCD34<sup>+</sup>CD38<sup>-</sup> population. Blood 2007;109:543-545.
11. Zijlmans JM, Visser JW, Kleiverda K, Kluijn PM, Willemze R, Fibbe WE. Modification of rhodamine staining allows identification of hematopoietic stem cells with preferential short-term or long term bone marrow-repopulating ability. Proc Natl Acad Sci U S A. 1995;92:8901-8905.
12. Beckman KB, Ames BN: Oxidative decay of DNA. J Biol Chem 1997;272:13300-13305.
13. Barzilai A, Yamamoto K. DNA damage responses to oxidative stress. DNA Repair 2004;3:1109-1115.
14. Altman SA, Zastawny TH, Randers-Eichorn L, Cacciottolo MA, Akman SA, Disdaroglu M, Rao G. Formation of DNA protein cross-links in cultured mammalian cells upon treatment with iron ions. Free Radic Biol Med 1995;19:897-902.
15. Cadet J, Delatour T, Douki T, Gasparutto D, Pouget JP, Ravanat JL. Sauvageo S: Hydroxyl radicals and DNA base damage. Mutat Res 1999;424:9-21.
16. Symington LS, Gautier J. Double-strand break end resection and repair pathway choice. Annu Rev Genet 2010 Nov 8 [Epub].
17. Kryston TB, Georgiev AB, Pissis P, Georgakilas AG. Role of oxidative stress and DNA damage in human carcinogenesis. Mutat Res 2011; Jan 7 [Epub].
18. Espejel S, Franco S, Rodriguez-Perales S, Bouffier SD, Cigudosa JC, Blasco MA. Mammalian Ku86 mediates chromosomal fusions and apoptosis caused by critically short telomeres. EMBO J 2002;21:2207-2219.
19. Nohl H. Generation of superoxide radicals as byproducts of cellular respiration. Ann Biol Clin 1994;52:199-204.
20. Vilenchik MM, Knudson AG: Endogenous DNA double strand breaks: production, fidelity of repair, and induction of cancer. Proc Natl Acad Sci USA 2003;100:12871-12876.
21. Floyd RA, West M, Henssley K: Oxidative biochemical markers; clues to understanding aging in long-lived species. Exp Gerontol 2001;36:619-640.
22. Marnett LJ, Riggins JN,West JD. Endogenous generation of reactive oxidants and electrophiles and their reactions with DNA and protein. J Clin Invest 2003;111:583-593.
23. Blagosklonny MV. Aging: ROS or TOR. Cell Cycle 2008;7:3344-3354.
24. Weinberger M, Mesquita A, Carroll T, Marks L, Yang H, Zhang Z, Ludovic P, Burhans, WC. Growth signaling promotes chronological aging in budding yeast by inducing superoxide anions that inhibit quiescence. 2010. Aging (Albany NY). 2010; 2:709-726.
25. Burhans WC, Weinberger M. DNA replication stress, genome instability and aging. Nucleic Acids Res 2007; 33;7545-7556.
26. Demidenko ZN, Zubova SG, Bukreeva EI, Pospelov VA, Pospelova TV, Blagosklonny MV. Rapamycin decelerates cellular senescence. Cell Cycle. 2009; 8:1888-1895.
27. Pospelova TV, Demidenko ZN, Bukreeva EI, Gudkov VA, Blagosklonny MV. Pseudo-DNA damage response in senescent cells. Cell Cycle 2009;8:4112-4118.
28. Leontieva OV, Blagosklonny MV. DNA damaging agents and p53 do not cause senescence in quiescent cells, while consecutive reactivation of mTOR is associated with conversion to senescence. Aging (Albany NY) 2010; 2:924-935.
29. Schoefield R. The relationship between the spleen colony-forming cell and the haemopoietic stem cell. Blood Cells 1978;4:7-25.
30. Jones DL, Wagers AJ. No place like home: anatomy and function of the stem cell niche. Nat. Rev. Mol. Cell Biol. 2008;9:11-21.
31. Mohyeldin A, Garzon-Muvdi T, Quinones-Hinojosa A. Oxygen in stem cell biology: A critical component of the stem cell niche. Cell Stem Cell 2010; 7:150-162.
32. Jing D, Wobus M, Poitz DM, Borinhauser M, Ehninger G, Ordemann R. Oxygen tension plays a critical role in the hematopoietic microenvironment in vitro. Haematologica 2011 Nov 4 (Epub).
33. Brahim-Horn MC, Pouyssegur J. Oxygen, a source of life and stress. FEBS Lett 2007;581:3582-3591.
34. Matsumoto A, Matsumoto S, Sowers AL, Koscielniak JW, Trigg NJ, Kuppusamy P, Mitchell JB, Subramanian S, Krishna MC, Matsumoto K. Absolute oxygen tension (pO2) in murine fatty and muscle tissue as determined by EPR. Magn Reson Med 2005;54:1530–1535.
35. Eliasson F, Jonsson JI. The hematopoietic stem cell niche: low in oxygen but a nice place to be. J Cell Physiol 2010;222:17–22.
36. Ezashi T, Das P, Roberts RM. Low O2 tensions and the prevention of differentiation of hES cells. Proc Natl Acad Sci USA 2005;102:4783–4788.
37. Yoshida Y, Takahashi K, Okita K, Ishisaka T, Yamanaka S. Hypoxia enhances the generation of induced pluripotent stem cells. Cell Stem Cell 2009;5:237–241.
38. De Filippis L, Delia D. Hypoxia in the regulation of neural stem cells. Cell Mol Life Sci 2011;68:2831-2844.
39. Rehn M, Olsson A, Reckzeh K, Diffner E, Carmeliit P, Landberg G, Cammenga J. Hypoxic induction of vascular endothelial growth factor regulates murine hematopoietic stem cell function in the low-oxygenic niche. Blood 2011;118:1534-1543.
40. Jing D, Wobus M, Potz DM, Borinhauser M, Ehninger G, Ordemann R. Oxygen tension plays a critical role in the hematopoietic microenvironment in vitro. Haematologica 2011 Nov 4. Epub.
41. Mazumdar J, O’Brien WT, Johnson RS, LaManna JC, Chavez JC, Klein PS, Simon MC. O2 regulates stem cells through Wnt/β-catenin signaling. Nature Cell Biol 2010;12:1007-1009.
42. Miharada K, Karlson G, Rehn M, Rorby M, Siva K, Cammenga J, Karlsson S. Chripto regulates hematopoietic stem cells as a hypoxic-niche-related factor through cell surface receptor GPR78. Cell Stem Cell 2011;9:330-344.
43. Wang J, Han F, Wu J, Lee SW, Chen CH, Wy CY, Yang WL, Gao Y, Zhang X, Jeong YS, Moten A, Samaniego F, Huang P, Liu Q, Zeng YX, Lin HK. The role of Skp2 in hematopoietic stem cell quiescence, pool size and self-renewal. Blood 2010 Sep 11 [Epub].
44. Bjorjorn CR, Cheung TH, Liu PV, Steeper KM, Rando TA. Notch signaling is necessary to maintain quiescence in adult muscle stem cells. Stem Cells 2011 Nov. 1 doi:1002/stem.773 [Epub].
45. Suda T, Takubo K, Semenza GL. Metabolic regulation of hematopoietic stem cells in the hypoxic niche. Cell Stem Cell 2011;9:298-310.
46. Zou P, Yoshihara K, Tai I, Shinmyozu K, Tsukahara F, Maru Y, Nakayama K, Nakayama KI, Suda T. p57Kip2 and p27Kip1 cooperate to maintain hematopoietic stem cell quiescence through interactions with Hsc70. Cell Stem Cell 2011;9:247-261.
47. Li L, Bhatia R. Stem cell quiescence. Clin Cancer Res 2011;17:493641.
48. Darzynkiewicz Z, Traganos F, Sharples T, Melamed MR. Lymphocyte stimulation: A rapid multiparameter analysis. Proc Natl Acad Sci USA 1976;73:2881-2884.
49. Tanaka T, Kajstura M, Halicka HD, Traganos F, Darzynkiewicz Z. Constitutive histone H2AX phosphorylation and ATM activation are strongly amplified during mitogenic stimulation of lymphocytes. Cell Prolif 2007;40:1-13.
50. Ratajczak MZ, Liu R, Maricz W, Blogowski W, Starzynska W, Wojakowski W, Zuba-Sarma E. Identification of very small embryonic/epiblast stem cells circulating in peripheral blood during organ/tissue injuries. Methods Cell Biol 2011;103:31-52.
51. Mantel C, Messina-Graham S, Broxmeyer HE. Upregulation of nascent mitochondrial biogenesis in mouse hematopoietic stem cells parallels upregulation of CD34 and loss of pluripotency: a potential strategy for reducing oxidative risk in stem cells. Cell Cycle 2010;9:2008-2017.
52. Darzynkiewicz Z. Guarding genome integrity in stem cells. Cell Cycle 2010;9:2271-2272.
53. E.A. Balazs, Editor. Hyaluronan. From Basic Science to Clinical Applications. Vols. 1, 2 and 3. PubMed, Edgewater, NJ. 2011.
54. Louderbough IMV, Schroeder JA. Understanding the dual nature of CD44 in breast cancer progression. Mol Cancer Res 2011;9:1573-1586.
55. Nilsson SK, Haylock DN, Johnston HM, Occhiodoro T, Brown TJ, Simmons PJ. Hyaluronan is synthesized by primitive hematopoietic cells, participates in their lodgment at the endostem following transplantation, and is involved in the regulation of their proliferation and differentiation in vitro. Blood, 2003;101:856-863.
56. Nilsson SK, Johnson HM, Coverdalwe JA. Spatial localization of transplanted hematopoietic stem cells: inferences for the localization of stem cells niches. Blood 2003;97:2293-2299.
57. Haylock DN, Nilsson SK. The role of hyaluronic acid in hematopoietic stem cell biology. Regen Med 2006;1:437-445.
58. Ellis SL, Grasser J, Jones SA, Borg J, Camenish T, Haylock D, Bertoncello I, Nilsson SK. The relationship between bone, hematopoietic stem cells, and vasculature. Blood 2011;118:1516-1524.
59. EllisSL, Williams B, Asquith S, Bertoncello I, Nilsson SK. An innovative triple immunogold method to investigate the hematopoietic stem cell niche in situ. Microsc Microanal 2009;15:403-414.
60. Preston M, Sherman LS. Neural stem cell niches: roles for the hyaluronan-based extracellular matrix. Front Biosci (Schol Ed) 2011;3:1165-1179.
61. Chen PY, Huang LL, Hsieh HJ. Hyaluronan preserves the proliferation and differentiation potentials of long-term cultured murine adipose-derived cells. Biochem Biophys Res Commun 2007;360:1-6.
62. Darzynkiewicz Z, Balazs EA. Effect of connective tissue intercellular matrix on lymphocyte stimulation. I. Suppression of lymphocyte stimulation by hyaluronic acid. Exp Cell Res 1971;66:113-123.
63. Pilarski LM, Pruski E, Wizniak J, Paine D, Seeberger K, Mant MJ, Brown CB, Belch AR. Potential role for the hyaluronan receptor RHAMM in mobilizing and trafficking of hematopoietic progenitor cells. Blood 1999;83:2818-2927.
64. Choudhary M, Zhang X, Stojkovic P, Hyslop L, Anyfantis G, Berbert M, Murdoch AP, Stojkovic M, Lako M. Putative role of hyaluronan and its ferated genes, HAS2 and RHAMM, in human early preimplantation embryogenesis and embryonic stem cell characterization. Stem Cells 2007;25:3045-3057.
65. Christophis C, Taubert I, Meseck GR, Schubert M, Grunze M, Ho AD, Rosenhahn A, Shear stress regulates adhesion and rolling of CD44+ leukemic and hematopoietic progenitor cells on hyaluronan. Biophys J 2011;101:585-593.
66. Shirvaikar N, Marquez-Curtis LA, Ratajczak MZ, Janowska-Wieczorek A. Hyaluronic acid and thrombin upregulate MT1-MMP through PI3K and Rac-1 signaling and prime the homing- related responses of cord blood hematopoietic stem/progenitor cells. Stem Cells Dev 2011;20:19-30.
67. Ramirez MA, Perecuesta E, Yanez-Mo M, Palasz A, Gutierrez-Adan A. Effect of long-term culture of mouse embryonic stem cells under low oxygen concentration as well as on glycosaminoglycan hyaluronan on cell proliferation and differentiation. Cell Prolif 2011;44:75-85.
68. Liu C-M, Yu C-H, Chang C-H, Hsu C-C, Huang LLH. Hyaluronan substratum holds mesenchymal stem cells in slow-cycling mode by prolonging G1 phase. Cell Tissue Res 2008;334:435-443.
69. Kim BS, Choi JS, Kim JD, Yeo TY, Cho YW. Improvement of stem cell viability in hyaluronic acid hydrogels using dextran microspheres. J Biomater Sci Polym Ed 2010;21:1701-1711.
70. Rossi CA, Faiibani M, Blaauw B, Pozzobon M, Figallo E, Reggiani C, VitielloL, Elvassore N, De Coppi P. In vivo tissue engineering of functional skeletal muscle by freshly isolated satellite cells embedded in a photopolymerizable hydrogel. FASEB J 2011;25:2296-2304.
71. Lei Y, Golgine S, Lam J, Segura T. The spreading, migration and proliferation of mouse mesenchymal stem cells cultured inside hyaluronic acid hydrogels. Biomaterials 2011;32:39-47.
72. Ratliff BB, Ghaly T, Brudnicki P, Yasuda K, Rajdev M, Bank M, Mares J, Hatzopoulos AK, Goligorsky MS. Endothelial progenitors encapsulated in bioartificial niches are insulated from systemic cytotoxicity and are angiogenesis competent. Am J Physiol Renal Physiol 2010;299:F178-F186.
73. Matrosova VY, Orlovskaya IA, Serobyan N, Khaldoyniadi SK. Hyaluronic acid facilitates the recovery of hematopoiesis following 5-fluorouracil administration. Stem Cells 2004;22:544-585.
74. Dalerba P, Dylla SJ, Park IK Liu R, Wang X, Cho RW, Hoey T, Gurney A, Huang EH, Simeoune DM, Shelton AA, Parmiani G, Castelli C, Clarke MF. Phenotypic characterization of human colorectal cancer stem cells. Proc Natl Acad Sci USA 2007;104:10158-10163.
75. Prince ME, Sivanandan R, Kaczorowski A, Wolf GT, Kaplan MJ, Dalerba P, Weissman IL, Clarke MF, Ailles LE. Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma. Proc Natl Acad Sci USA 2007;104:973-978.
76. Li C, HeeDG, Dalerba P, Burtan CF, Zhang L, Adsay V, Wicha M, Clarke MF, Simeone DM. Identification of pancreatic cancer stem cells. Cancer Res 2007;67:1030-1037.
77. Stern, R (Editor) Hyaluronan in Cancer Biology. 2009, Elsevier/Academic Press, Amsterdam.
78. Baco Z, Nagy H, Goda K, Bene L, Fenyesi F, Matko J, Szabo G. Raft and cytoskeleton association of an ABC transporter: P-glycoprotein. Cytometry A, 2004;61A:106-116.
79. Misra S, Ghatak S, Toole BP. Regulation of MDR1 expression and drug resistance by a positive feedback loop involving hyaluronan, phosphoinositide-3-kinase and ErbB2. J Biol Chem 2005;20319-29315.
80. Mista S, Ghatak S, Zoltan-Jones A, Toole BP. Regulation of multi-drug resistance in cancer cells by hyaluronan. J Biol Chem 278;25285-25288.
81. Slomiany MG, Toole BP. Hyaluronan-CD44 interactions and chemoresistance in cancer cells. In Hyaluronan in Cancer Biology, R. Stern (Ed) 2009, Elsevier/Academic Press, Amsterdam.
82. Griffiths HR, Dunston CR, Bennett SJ, Grant MM, Phillips DC, Kitas GD. Free radicals and redox signaling in T-cells during inflammation and aging. Biochem Soc Trans 2011;39:1273-1278.
83. Spooner R, Yilmaz O. The role of reactive-oxygen-species in microbial persistence and inflammation. Int J Mol Sci 2011;12:334-352.
84. Naik E, Dixit VM. Mitochondrial reactive oxygen species drive proinflammatory cytokine production. J Exp Med 2011;208:417-420.
85. Tschopp J. Mitochondria: Sovereign or inflammation. Eur J Immunol 2011;41:1196-1202.
86. Yang C, Ling H, Zhang M, Yang Z, Wang X, Zeng F, Wang C, Feng J. Oxidative stress mediates chemical hypoxia-induced injury and inflammation by activating NF-kB-COX-2 pathway in HaCaT cells. Mol Cells 2011;31:531-538.
87. Martinon F. Signaling by ROS drives inflammasome activation. Eur J Immunol 2010;40:616-619.
88. Balazs E, Davies J, Phillips G, Young M. Transient intermediates in the radiolysis of hyaluronic acid. Radiat Res 1967;31:243-255.
89. Albertini R, Passi A, Abuja PM, De Luca G. The effect of glycosaminoglycans and proteoglycans on lipid peroxidation. Int J Mol Med 2000;6:126-136.
90. Balazs EA. Viscosupplementation for treatment of osteoarthritis: from initial discovery to current status and results. Surg Technol Int 2004;12:278-289.
91. Mytar B, Siedlar M, Woloszyn M, Colizzi V, Zembala M. Cross-talk between human monocytes and cancer cells during reactive oxygen intermediates generation: the essential role of hyaluronan. Int J Cancer 2001;94:727-732.
92. Yu H, Zhou Z, Kolosov VP, Perselman JM. Role of hyaluronan and CD44 in reactive oxygen species-induced mucus hypersecretion. Mol Cell Biochem 2011;352:65-75.
93. Poulton T, Dutoit M, Joly F, Warnet JM, Rat P. High molecular weight hyaluronan decreases UVB-induced apoptosis and inflammation in human epithelial corneal cells. Mol Vis 2009;15:577-583.
94. Riehl TE, Foster L, Stenson WF. Hyaluronic acid is radioprotective in the intestine through a TLR-4 and COX-2 mediated mechanism. Am J Physiol Gastrointest Liver Physiol 2011; Oct 28 (Epub).
95. Balazs EA, Denlinger J. Viscosupplementation: a new concept in the treatment of osteoarthritis. J Rheumatol Suppl 1993;39:3-9.
96. Campo GM, Avenoso A, Nastasi G, Micali A, Prestipino V, Vaccaro M, D’Ascola A, Calatroni A, Campo S. Hyaluronan reduces inflammation in experimental arthritis by modulating TLR-2 and TLR-4 cartilage expression. Biochem Biophys Acta 2011;1812:1170-1181.
97. Curran MP. Hyaluronic acid (Supartz®): a review of its use in osteoarthritis of the knee. Drugs Aging 2010;27:925-941.
98. Stenson WF. Hyaluronic acid and intestinal inflammation. Curr Opin Gastroenterol 2010;26:85-87.
99. Heldin B, Karousoe E, Bernert B, Porsch H, Nishitsuka K, Skandalis SS. Importance of hyaluronan-CD44 interactions in inflammation and tumorigenesis. Connect Tiss Res 2008;49:215-218.
100. Shimizu C, Yoshioka M, Coutts FL, Hardwood FL, Kubo T, Hirasa Y, Amiel D. Long-term effects of hyaluronan on experimental osteoarthritis in the rabbit knee. Osteoarthritis Cartilage 1998;6:1-9.
101. Balazs EA, Davies JV, Phillips GO, Scheufele DS. Polyanions and their complexes. 3. Reactions of heparin, hyaluronic acid, sodium poly(styrenesulphonate), and sodium carboxymethyl-cellulose with hydroxyl radicals and hydrated electrons. J Chem Soc Perkin 1 1968;12:1420-1423.
102. Grishko V, Xu M, Ho R, Mates A, Watson S, Kim JT, Wilson GL, Pearsall AW 4th. Effect of hyaluronic acid on mitochondrial function and mitochondria-driven apoptosis following oxidative stress in human chondrocytes. J Biol Chem 2009;284:9132-9139.
103. Sato T, Takahashi T, Ide H, Fukushima T, Tabata M, Sekina F, Kobayashi K, Nagishi M, Niwa Y. Antioxidant activity of synovial fluid, hyaluronic acid, and two subcomponents of hyaluronic acid. Synovial fluid scavenging effect is enhanced in rheumatoid arthritis patients. Arthritis Rheum 1988;31:63-71.
104. Greenwald RA, Moy WW. Effect of oxygen-derived free radicals on hyaluronic acid. Arthritis Rheum 1980;23:455-463.
105. Balazs EA, Darzynkiewicz Z. The effect of hyaluronic acid on fibroblasts, mononuclear phagocytes and lymphocytes. In: Biology of the Fibroblast, Papers of the Symposium held in Turku, Finland, 1972. Kulonen E and Pikkarainen J (eds.) Academic Press, London, pp. 237-252, 1973.
106. Suzuki Y, Yamaguchi T. Effects of hyaluronic acid on macrophage phagocytosis and active oxygen release. Agents Action 1993;38:332-337.
107. Fukuda K, Takayama M, Ueno M, Oh M, Asada S, Kumanu F, Tanaka S. Hyaluronic acid inhibits interleukin-1-induced superoxide anion in bovine chondrocytes. Inflam Res 1997;46:114-117.
108. Soltes L, Mendichi R, Kogan G, Schiller J, Stankovska M, Amhold J. Degradative action of reactive oxygen species on hyaluronan. Biomacromolecules 2006;7:659-668.
109. Chen WY, Abatangelo G. Functions of hyaluronan in wound repair. Wound Repair Regen 1997;5:79-89, 1999.
110. Haider AS, Grabarek J, Eng B, Pedraza P, Ferreri NR, Balazs EA, Darzynkiewicz Z. In vitro wound healing analyzed by laser scanning cytometry. Accelerated healing of epithelial cell monolayers in the presence of hyaluronan. Cytometry A 2003; 53A:1-8.
111. Zhao H, Tanaka T, Mittlitski V, Heeter J, Balazs EA, Darzynkiewicz Z. Protective effect of hyaluronate on oxidative DNA damage in WI-38 and A549 cells. Int J Oncol 2008;32:1159-1169.
112. Tanaka T, Halicka HD, Traganos F, Darzynkiewicz Z. Phosphorylation of histone H2AX on Ser 139 and activation of ATM during oxidative burst in phorbol ester-treated human leukocytes. Cell Cycle 2006;5:2671-2675.

113. Halicka HD, Mitiliski V, Heeter J, Balazs EA, Darzynkiewicz Z. Attenuation of the oxidative burst induced DNA damage in human leukocytes by hyaluronate. Int J Mol Med 2009;23:695-699.

114. Tanaka T, Halicka HD, Huang X, Traganos F, Darzynkiewicz Z. Constitutive histone H2AX phosphorylation and ATM activation, the reporters of DNA damage by endogenous oxidants. Cell Cycle 2006;5:1940-1945.

115. Zhao H, Tanaka T, Halicka HD, Traganos F, Zarebski M, Dobrucki J, Darzynkiewicz Z. Cytometric assessment of DNA damage by exogenous and endogenous oxidants reports the aging-related processes. Cytometry A 2007; 71A:905-914.

116. Campo GM, Avenoso A, Campo S, D’Ascola A. Ferlazzo AM, Calatroni A. Reduction of DNA fragmentation and hydroxyl radical production by hyaluronic acid and chondroitin-4-sulphate in iron plus ascorbate-induced oxidative stress in fibroblast cultures. Free Radic Res 2004;38:601-611.

117. Parmegiani L, Cognigni GE, Bernardi S, Trollo E, Ciampaglia W, Filiciori M. “Physiological ICIS”: hyaluronic acid (HA) favors selection of spermatozoa without DNA fragmentation and with normal nucleus, resulting in improvement of embryo quality. Fertil Steril 2010;93:598-604.

118. Razavi SH, Nasr-Esfahani MH, Deemeh MR, Shayesteh M, Tavalaee M. Evaluation of beta and HA-binding methods for selection of spermatozoa with normal morphology, protamine content and DNA integrity. Andrologia 2010;42:13-19.

119. Hijji M, Creemers E, Cox A, Janssen M, Vanheusden E, Van der Elst J, Ombelet W. Relationship between hyaluronic acid binding assay and outcome in ART: a pilot study. Andrologia 2010;42:291-296.

120. Yagci A, Murk W, Stronk J, Huszar G. Spermatozoa bound to solid state hyaluronic acid show chromatin structure with high DNA chain integrity: an acridine orange fluorescence study. J Androl 2010;31:566-572.

121. Petersen CG, Massaro FC, Mauri AL, Oliveira JB, Baruffi RL, Franco JG Jr. Efficacy of hyaluronic acid binding assay in selecting motile spermatozoa with normal morphology at high magnification. Reprod Biol Endocrinol 2010 Dec 3, 8:149.

122. Evenson DP, Darzynkiewicz Z, Melamed MR. Relation of mammalian sperm chromatin heterogeneity to fertility. Science 1980;210:1131-1133.

123. Gorczyca W, Traganos F, Jesionowska H, Darzynkiewicz Z. Presence of DNA strand breaks and increased sensitivity of DNA in situ to denaturation in abnormal human sperm cells. Analogy to apoptosis of somatic cells. Exp Cell Res 1993;207:202-205.

124. Ranganathan S, Ganguly AK, Datta K. Evidence for the presence of hyaluronan binding protein on spermatozoa and its possible involvement in sperm function. Mol Reprod Dev 1994;38:69-76.

125. Furnas CC, Valcarcel A, Dulout FN, Errecalde AL. The hyaluronic acid receptor (CD44) is expressed in bovine oocytes and early stage embryos. Theriogenology 2003;60:1633-1644.

126. Borg N, Holland M. The effect of glycosaminoglycans on rat gametes in vitro and the associated signal pathway. Reproduction. 2008;135:311-319.

127. Hua Q, Knudson CB, Knudson W. Internalization of hyaluronan by chondrocytes occurs via receptor-mediated endocytosis. J Cell Sci 1993;106:365–375.

128. Embry JJ, Knudson W. G1 domain of aggrecan cointernalizes with hyaluronan via a CD44-mediated mechanism in bovine articular chondrocytes. Arthritis Rheum 2003;48:3431-3441.

129. Ouasti S, Kingham PJ, Terenghi G, Tirrell N. The CD44/integrins interplay and the significance of receptor binding and re-presentation in the uptake of RGD-functionalized hyaluronic acid. Biomaterials 2012;33:1120-1134.

130. Ueno S, Yoshida N, Nijmura S. Amount of hyaluronan produced by mouse oocytes and role of hyaluronan in enlargement of the perivitelline space. J Reprod Dev 2009;55:496-501.

131. Kan FW. High-resolution localization of hyaluronic acid in the golden hamster oocyte-cumulus complex by use of a hyaluronidase-gold complexes. Anat Res 1990;228:370-382.

132. Saito H, Kaneko T, Takahashi T, Kawachiya S, Saito T, Hiroi M. Hyaluronan in follicular fluids and fertilization of oocytes. Fertil Steril 2000;74:1148-1155.

133. Yokoo M, Kimura N, Abe H, Sato E. Influence of hyaluronan accumulation during cumulus expansion on in vitro porcine oocyte maturation. Zygote 2008;16:309-314.

134. Yokoo M, Kimura N, Sato E. Induction of oocyte maturation by hyaluronan-CD44 interaction in pigs. J Reprod Dev 2010;56:15-19.

135. Su L, Mruk DD, Cheng CY. Drug transporters, the blood-testis barrier, and spermatogenesis. J Endocrinol 2011;208:207-223.

136. Kubota H, Avarbock MR, Brinster RL. Spermatogonial stem cells share some, but not all, phenotypic and functional characteristics with other stem cells. Proc Natl Acad Sci USA 2003;100:6477-6492.

137. Scaldaferreri ML, Fera S, Grisanti L, Sanchez M, Stefanini M, De Felici M, Vicini E. Identification of side population cells in mouse primordial germ cells and prenatal testis. Int J Dev Biol 2011;55:209-214.

138. Balogh GT, Illes J, Szekely Z, Forrai E, Gere A. Effect of different metal ions on the oxidative damage and antioxidant capacity of hyaluronic acid. Arch Biochem Biophys 2003;410:76-82.

139. Nagy L, Yamashita S, Yamaguchi S, Sipos P, Wakita H, Nomura M. The local structures of Cu(II) and Zn(II) complexes of hyaluronate. J Inorg Biochem 2000;72:49-55.

140. Koppenol WH. The Haber-Weiss cycle - 70 years later. Redox Rep 2002;7:55-57.

141. Lloyd RV, Hanna PM, Mason RP. The origin of the hydroxyl radical oxygen in the Fenton reaction. Free Rad Biol Med 1997;22:885-888.

142. Draf F, Bauerova K, Valachova K, Ponist S, Mihalova D, Juranek I, Boldyrev A, Hrabarova E, Soltes L. Carnosine inhibits degradation of hyaluronan induced by free radical processes in vitro and improves the redox imbalance in adjuvant arthritis in vivo. Neuro Endocrinol Lett 2010; 31, Suppl 2:96-100.

143. Hutadiok N, Smith MM, Ghosh P. Effect of hydrogen peroxide on the metabolism of human rheumatoid and osteoarthritic synovial fibroblasts in vitro. Ann Rheum Dis 1991;50:219-226.

144. Hong Y, Cervantes RB, Tichy E, Tischfield JA, Stanbrook PJ. Protecting genomic integrity in somatic cells and embryonic stem cells. Mutat Res 2007;614:48-55.
146. Maynard S, Swistowska AM, Lee JW, Liu Y, Liu ST, Da Cruz AB, Rao M, de Souza-Pinto NC, Zeng X, Bohr VA. Human embryonic stem cells have enhanced repair of multiple forms of DNA damage. Stem Cells 2008;26:2266-2274.

147. FillionTM, Qiao M, Ghule PN, Mandeville M, van Wijnen AJ, Stein JL, Lian JB, Altieri DC, Stein GS. Survival responses of human embryonic stem cells to DNA damage. J Cell Physiol 2009;220:586-592.

148. Priott SC, Freeland A, Kudla A. Cell heterogeneity in the small intestinal crypt and maintenance of genome integrity. Stem Cells 2010;28:1250-1259.

149. Parrinello S, Samper E, Krtolica A, Goldstein J, Melov S, Campisi J. Oxygen sensitivity severely limits the replicative lifespan of murine fibroblasts. Nat Cell Biol 2003;5:741-747.

150. Basutti RA, Rubio M, Dolle ME, Campisi J, Vijg J. Oxygen accelerates the accumulation of mutations during the senescence and immortalization of murine cells in culture. Aging Cell 2003;2:287-294.

151. Balazs, EA. Hyaluronic acid and matrix implantation. A report on the biological activity and therapeutic use of hyaluronic acid. 1971. Published by Biotrics, Inc., Arlington, MA (www.matrixbiologyinstitute.org).

152. Balazs EA, Mitlitski I, Heeter J. In vitro cell behavior on hyaluronan fluids and gels. Proceedings of the 8th International Conference on Hyaluronan, Kyoto, Japan, 2010. (www.matrixbiologyinstitute.org).

153. Naka K, Hirao A. Maintenance of genomic integrity in hematopoietic stem cells. Int J Hematol 2011;93:434-439.

154. Suda T, Takubo K, Semenza GL. Metabolic regulation of hematopoietic stem cells in the hypoxic niche. Cell Stem Cell 2011;9:298-310.

155. Hao Y, Cheng D, Ma Y, Zhou W, Wang Y. The relationship between oxygen concentration, reactive oxygen species and the biological characteristics of human bone marrow hematopoietic stem cells. Transplant Proc 2011;43:2755-2761.

156. Sardina JL, Lopez-Ruano G, Sanchez-Sanchez B, Llanillo M, Hernandez-Hernandez A. Reactive oxygen species: are they important for haematopoiesis? Crit Rev Oncol Hematol 2011; Apr 18 Epub.

157. Zhu H, Mitsuhashi N, Klein A, Barsky LW, Weinberg K, Barr ML, Demetriou A, Wu GD. The role of hyaluronan receptor in mesenchymal stem cell migration in the extracellular matrix. Stem Cells 2006;24:928-935.

158. Grunert M, Nurcombe V, Cool SM. Stem cell fate decisions: the role of heparin sulfate in the control of autocrine and paracrine signals. Curr Stem Cell Res Ther 2008;31:1-8.

159. Breen EC. VEGF in biological control. J Cell Biochem 2007;102:1358-1367.