

DNA damage repair response in mesenchymal stromal cells: From cellular senescence and aging to apoptosis and differentiation ability

Peer-reviewed author version

Banimohamad-shotorbani, Behnaz; KAHROBA, Housman; Sadeghzadeh, Hadi; WILSON, David; Maadi, Hamid; Samadi, Nasser; Hejazi, Mohammad Saeid; Farajpour, Hekmat; Onari, Behzad Nemati & Sadeghi, Mohammad Reza (2020) DNA damage repair response in mesenchymal stromal cells: From cellular senescence and aging to apoptosis and differentiation ability. In: Ageing Research Reviews, 62 (Art N° 101125).

DOI: 10.1016/j.arr.2020.101125

Handle: <http://hdl.handle.net/1942/32880>

DNA damage and repair response in mesenchymal stem cells: from cellular senescence and aging to apoptosis and differentiation ability

Behnaz banimohamad-shotorbani^{1,7†}, Houman Kahroba^{2,3,4,12*†}, Hadi Sadeghzadeh^{2,7†}, David M Wilson III⁸, Hamid Maadi⁹, Nasser Samadi^{4,5}, Mohammad Saeid Hejazi^{3,4,6}, Hekmat Farajpour¹⁰, Behzad Nemati Onari¹¹

1. Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.
2. Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.
3. Molecular Medicine Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.
4. Department of Molecular Medicine, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran.
5. Department of Biochemistry, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran.
6. Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.
7. Tissue Engineering Department, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran.
8. Hasselt University, Biomedical Research Institute, Diepenbeek, Belgium.
9. Department of Medical Genetics, Faculty of Medicine and Dentistry, University of Alberta.
10. Department of Tissue Engineering and Applied Cell Sciences, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
11. Neurosciences Research Center, Imam Reza Hospital, Tabriz University of Medical Sciences, Tabriz, Iran.
12. Students Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran.

*Corresponding author:

Houman Kahroba: houman.kahroba@yahoo.com

Department of Molecular Medicine, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran

Tel: +989369411587. Fax: +984133377643

†: These authors are contributed equally to this study.

Abstract

Mesenchymal stem cells (MSCs) are multipotent stromal cells originating from several adult tissues and sources, including bone marrow, adipose tissue, skin, placenta, umbilical cord blood, amniotic fluid, and peripheral blood. MSCs' secretome has the ability to induce proliferation, differentiation, chemo-attraction, anti-apoptosis, and immunomodulation activities in stem cells. Moreover, MSCs can recognize tissue damage caused by drugs, radiation (e.g., Ultraviolet, infra-red) and oxidative stress, typically responding in one of two ways. First, MSCs can differentiate into particular cell lineages to preserve tissue homeostasis, or second, they can release a regenerative secretome to activate tissue repairing mechanisms. The continued presence of MSCs in a quiescent phase (G0) can increase the incidence and accumulation of various forms of genomic modifications, particularly upon environmental injury. Thus, dysregulated DNA repair pathways can predispose MSCs to senescence or apoptosis, reducing their stemness and self-renewal properties. In particular, DNA damage can impair telomere replication, activating DNA damage checkpoints to maintain MSC function. In this review, we aim to summarize the role of DNA damage and associated repair responses in MSC senescence, differentiation, programmed cell death, and aging.

Keywords

Malignancy, Base Excision Repair, Nucleotide Excision Repair, Homologous Recombination, Non-Homologous End Joining, Mismatch Repair

1. Introduction

Adult stem cells (ASCs) can promote tissue repair and regeneration upon injury and sustain inter-cellular heterogeneity for physiological homeostasis. The number and function of stem cells (SCs), in addition to their compartmentalization, contribute to the pathophysiological status of tissues (Vitale et al., 2017a). We know SCs for their self-renewal capacity by infinite asymmetric divisions and their differentiation to cells of various lineages based on their origin and differentiation capacity, their so-called stemness. SCs are categorized as totipotent, pluripotent, multipotent, oligopotent, and unipotent cells. These classifications are according to plasticity and tissue regeneration potency of SCs. Endogenous and exogenous damages on DNA can cause genetic lesions that may challenge cell survival and function. The significant roles of DNA in living organisms necessitate precise control of DNA damage repair mechanisms through recruitment of repair factors to damage sites and the activation of checkpoint regulators to attenuate or arrest cell-cycle progression. These mechanisms, which are known collectively as the DNA damage response (DDR), can execute full repair or promote the elimination of damaged cells to protect host organisms against different malignancies (Bielak-Zmijewska et al., 2018). Adult stem and progenitor cells in various tissues are generally equipped with several regulatory mechanisms to guarantee genome integrity and tissue homeostasis. Dysregulation of DNA repair pathways in SCs can reduce tissue regeneration capacity by limiting the self-renewal and differentiation properties of SCs and by inducing senescence or apoptosis in these cells (Weeden and Asselin-Labat, 2018).

Mesenchymal stem cells (MSCs) and Mesenchymal stromal cells are applied as equal terms in different studies. Here in, we focus on the MSCs that are located within the stroma of adult organs, although these cells are present in a wide range of tissue sources (Srinivasan et al., 2019). The secretome of MSCs contains tissue repairing elements that play an essential role in regulating local and remote progenitor and SC function. In response to tissue damage, MSCs can release factors that activate tissue repair mechanisms or that direct differentiation of SCs into certain cell lineages (Fitzsimmons et al., 2018; Moravej et al., 2019; Xi et al., 2013). In the present review, we summarize the contributions of the DDR in MSC senescence and apoptosis and highlight its pathophysiological relevance.

2. Mesenchymal stem cells

The International Society for Cell & Gene Therapy (ISCT) argues against the equality or interchangeability of the term MSC with mesenchymal stromal cell. ISCT defines MSCs as a heterogeneous population of stromal cells that

are capable of self-renewal and tri-lineage differentiation (to osteoblast, adipocyte, and chondrocyte). These cells are plastic-adherent under routine culture conditions and express CD73, CD90, and CD105 markers on their surface, but not CD11b, CD14, CD19, CD34, CD45, CD79a, and HLA class II markers (Pashoutan Sarvar et al., 2018; Viswanathan et al., 2019). Different sources have been reported for the isolation of MSCs, including bone marrow (BM), trabecular bone, periosteum, synovium, muscle, adipose tissue, pancreas and thymus, Wharton's jelly, dental pulp, peripheral blood, skin, lungs, amniotic fluid, chorionic villi, menstrual blood, umbilical cord blood, placenta, and breast milk (Eleuteri and Fierabracci, 2019; Hou et al., 2013; Moravej et al., 2019; Wong, 2011).

Local tissue damage can be sensed by MSCs, resulting in the secretion of specific factors to support tissue repair and regeneration (Fitzsimmons et al., 2018; Xi et al., 2013). The MSC secretome contains a significant amount of tissue repairing elements, including interleukins (e.g. IL-6 and IL-8), chemokines (e.g. CCL2, CCL5 and CXCL8), and soluble factors (e.g., epidermal growth factor receptor ligands and hepatocyte growth factor), which collectively can induce progenitor and SC proliferation, differentiation, and chemo-attraction (Broekman et al., 2016; Weyand and Eberle, n.d.). Moreover, these tissue-repairing elements can activate the anti-fibrosis, anti-apoptosis, and angiogenesis pathways in these cells (Srinivasan et al., 2019).

MSCs have limited self-renewal, multipotency, and differentiation capability in contrast to pluripotent SCs. However, easy isolation and lower tumorigenicity of MSCs make them a much safer source for cell therapies (Li et al., 2017a; Timari et al., 2017). After systemic injection of MSCs, these cells can migrate into the various target tissues with the capacity to differentiate into cartilage, muscle, bone, skin, heart, liver, lung, intestine, endothelial, skeletal muscle, neuron, and fat cells (Aqmasheh et al., 2017; Wilson et al., 2010). Mesenchymal stromal cells' secretome contains high amounts of bio-functional elements, including anti-inflammatory cytokines. These elements can boost local trophic and immunomodulatory activities to suppress innate and adaptive immune responses (Caplan, 2009; Ferreira et al., 2018). MSCs are continually being challenged by aging and genotoxic threats, which may result in losing their differentiation ability and cause senescence (Wilson et al., 2010).

3. Effect of DNA damage in MSCs

In contrast to somatic cells, which typically undergo terminal differentiation, SCs can survive and duplicate for an extended period, which can increase the possibility of incidence and accumulation of mutations in these cells (Mani et al., 2019). Chemical reactions between DNA and active molecules, including intracellular reactive oxygen species

(ROSs), can cause a wide range of DNA damage (Chatterjee and Walker, 2017). The main mechanisms of endogenous DNA-damage include hydrolysis, deamination, s-adenosylmethionine alkylation, and ROS-mediated oxidation. In some cases, these damages can serve as mutagenic templates or can distort the DNA double helix, resulting in adverse consequences during transcription or replication. For instance, single base alterations, such as cytosine to uracil and guanine to 8-oxoguanine, can cause mutagenesis and impair gene functionality, DNA binding sites or epigenetic patterns. Exogenous DNA-damaging agents can be divided into two main groups: a) physical agents, such as UV-radiation that produces cyclobutane pyrimidine dimers and 6-4 pyrimidine-pyrimidone (6-4PPs) (Osakabe et al., 2015), and b) chemical agents, such as cisplatin, a therapeutic compound that induces DNA intra- and inter-strand crosslinks. Furthermore, some chemicals, such as polycyclic aromatic hydrocarbons and malondialdehyde-related pyrimidopurinones, can introduce bulky DNA adducts as either endogenous or exogenous agents (Vitale et al., 2017b). A comprehensive list of genotoxic mechanisms and agents is provided in Table 1.

MSCs have been reported to both lose and improve their differentiation ability with age. Specifically, the ability of MSCs to differentiate to chondrogenic and osteogenic cells gradually reduces during aging, whereas aging increases the rate of differentiation to adipocytes in MSCs. The prolonged culture of MSCs results in the accumulation of chromosomal abnormalities followed by loss of self-renewal ability (Benameur et al., 2015; Wilson et al., 2010). Improper function of DNA repair mechanisms that can arise during long-term culture of MSCs can activate DDR pathways. However, the extent of DNA damage can eventually exceed the DDR system capacity, ultimately resulting in loss of differentiation capability and cellular senescence. P16 (c-20), phosphorylated-p53 (at Ser15), and p21Waf1/Cip1 are molecules associated with the DDR in late passages of MSCs (Alves et al., 2010). In vitro experiments have confirmed that human MSCs (hMSCs) are highly sensitive to the accumulation of DNA damage. For instance, the elevated generation of oxidative stress-related molecules as DNA-damaging agents can induce senescence and growth arrest in hMSCs. Moreover, the lack of DDR mechanisms in hMSCs leads to a loss of differentiation capacity, which negatively affects their potential clinical utility (Alves et al., 2010).

Table 1. List of endogenous and exogenous damaging mechanisms and agents.

Endogenous threats	Ref
Error-prone (non-proof-reading) DNA polymerases (α, β, σ, γ, λ, REV1, ζ, η, ι, κ, θ, ν, μ, Tdt and PrimPol)	(Loeb and Monnat, 2008)
Topoisomerases (TOP I, TOP II, TOP III; 7 TOP)	(Pommier et al., 2006)
Spontaneous base deamination (e.g., uracil)	(Chatterjee and Walker, 2017)

Spontaneous base hydrolysis (AP sites)	(Lindahl and Barnes, 2000)
ROS (range of oxidative DNA lesions, e.g., 8-oxoguanine)	(Henle and Linn, 1997)
Methylation (e.g., O6-methylguanine)	(O'Driscoll et al., 1999)
Exogenous threats	Ref
Ionizing radiation (alpha, beta, gamma, neutrons, X-ray)	(Desouky et al., 2015)
UV radiation	(Kiefer, 2007)
Alkylating agents (dietary components, tobacco smoke, biomass burning, industrial processing, and chemotherapeutic agents)	(Chatterjee and Walker, 2017)
Aromatic amines (cigarette smoke, fuel, coal, industrial dyes, pesticides, and everyday high-temperature cooking)	(Skipper et al., 2010; Sugimura, 1986)
Polycyclic aromatic hydrocarbon (tobacco smoke, automobile exhaust, charred food and incomplete combustion of organic matter and fossil fuels)	(Harvey, 1991; Yu, 2002)
Reactive electrophiles (4-nitroquinoline 1-oxide, N-nitrosamines)	(Chatterjee and Walker, 2017)
Toxins (Aflatoxins)	(Ames et al., 1990; Bennett and Klich, 2003)
Environmental stress (extreme heat or cold, hypoxia, and oxidative stress)	(Ejtehadifar et al., 2016; Gafter-Gvili et al., 2013; Luoto et al., 2013; Neutelings et al., 2013; Skipper et al., 2010)
Cosmetic components (butylparaben and bisphenol A), food preserves (sodium benzoate, potassium benzoate, and potassium sorbate), food additives (citric acid, phosphoric acid, brilliant blue, and sunset yellow)	(Chatterjee and Walker, 2017)

4. Role of DNA damage in stemness and differentiation of MSCs

Irradiation can trigger ROS accumulation in cells and cause DNA damage and eventually lead to the loss of stemness in MSCs. BM-MSCs show a different response upon exposure to low or high dose radiation. Low dose radiation can induce senescence, a defense response against tumorigenesis, and reduce the stemness of MSCs by attenuating autophagy activity (Alessio et al., 2015). Autophagy is an active mechanism to maintain the stemness of MSCs by decreasing ROS accumulation and DNA damage. Starvation and rapamycin (as a macrolide compound) can activate the autophagy process. Activation of autophagy has been shown to serve as a defense mechanism against irradiation damage to protect the stemness of MSCs (Hou et al., 2013). Additionally, silencing of Retinoblastoma protein 1 (RB1) in MSCs prior to X-ray irradiation can reduce cell proliferation and promote the onset of senescence (Alessio et al., 2017). High-dose photon and carbon ion radiation can induce DNA double-strand breaks (DSBs) in MSCs and arrest cell cycle at the G2 phase without affecting apoptosis (Nicolay et al., 2015). Exposure of MSCs to DNA-damaging

agents not only affects their stemness, viability and proliferation, but also leads to a significant decline in their differentiation ability (Alessio et al., 2017). For instance, chemotherapy can adversely affect the differentiation capacity of SCs through the induction of severe DNA damage (Asumda and Chase, 2011a; Cruet-Hennequart et al., 2012).

Mammalian target of rapamycin (mTOR) signaling pathway plays a vital role in cell metabolism, growth, proliferation, and survival. DNA damage, hypoxia, and energy stress can induce the expression of the DNA Damage Inducible Transcript 4 (DDIT4) gene (Ellisen et al., 2002; Gharibi et al., 2016; McGhee et al., 2009). Several studies have suggested a significant role for DDIT4 in differentiation capability, pluripotency gene expression, and regulation of the mTOR signaling pathway in MSCs. DDIT4 can regulate various biological functions of MSCs by negatively regulating the mTOR signaling pathway in response to hypoxic conditions and nutrient restriction. High expression levels of DDIT4 driven by DNA damage accumulation can decrease the differentiation ability, yet maintain the stemness of MSCs (Gharibi et al., 2016). We note that prolonged culture of MSCs can also alter the methylation pattern of DNA at specific CpG sites, an outcome that affects the expression of differentiation-associated genes, such as RUNX Family Transcription Factor 3 (RUNX3) (Bork et al., 2010).

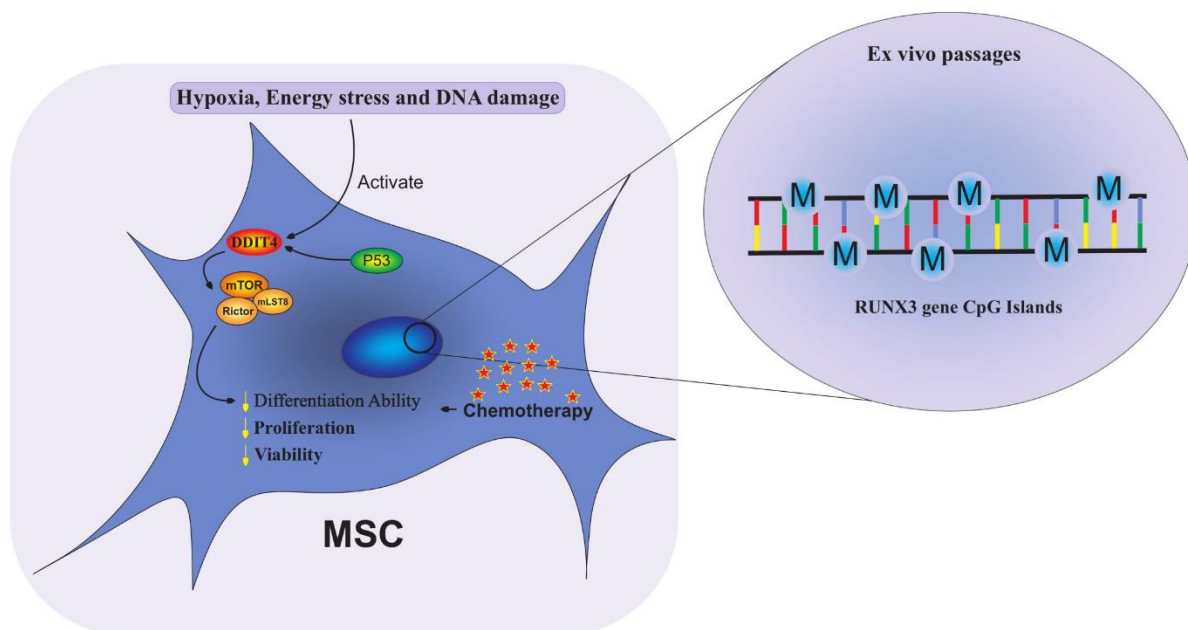


Figure1. MSCs DNA damage and differentiation ability. Hypoxia, energy stress, and DNA damage is the main factor in attenuating MSCs' differentiation ability. DNA damage can activate DDIT4, which subsequently alters the mTOR signaling pathway to decrease MSCs' differentiation ability. Also, P53 can upregulate DDIT4 expression in a P-53-depended manner. Chemotherapy agents are also able to reduce the MSCs' differentiation ability by inducing DNA damage. For instance, in ex-vivo studies, the lower capacity of differentiation is observed through the induction of methylation on CpG islands of the RUNX3 gene.

5. DNA damage in senescence and apoptosis of MSCs

DDR proteins are responsible for the detection of DNA damage to mediate appropriate cellular responses toward repair, senescence or apoptosis (Lombard et al., 2005; Roos and Kaina, 2006). Senescence is a unique state of durable cell-cycle arrest, which can be induced by various cellular stresses, including DNA damage accumulation. This process is one of the critical defense mechanisms to avoid malignancy in mammalian cells. Although senescent cells are non-replicative, they are metabolically active, and in rare conditions, these cells can redirect to replication-competent cells. Generally speaking, aging and cytogenetic stresses can direct MSCs towards senescence rather than apoptosis. Apoptosis is a selective hemostatic response to remove MSCs without self-renewal ability. Importantly, senescence in ASCs, in general, and MSCs, in particular, can reduce regeneration and repairing activities of SCs in tissues (He and Sharpless, 2017; Li et al., 2017b).

The main stresses that induce cellular senescence are dysfunctional telomeres, genomic lesions, disorganized chromatin structures, and active mitogenic signaling pathways. For example, when telomeres have reached a critically short length or DNA DSBs have become excessive, specific DDR pathways direct senescence-induced growth arrest (Turinetti et al., 2016). The stressor-derived DNA damage can be detected by specific DDR proteins, including Ataxia Telangiectasia Mutated (ATM) and p53 tumor suppressor, to inhibit cell cycle progression. Histone deacetylase inhibitors are known as potent activators of DDR proteins (e.g., ATM and p53 tumor suppressor) that direct cellular senescence (Munro et al., 2004; Ogryzko et al., 1996). Overall, genotoxic stress can induce replication arrest and the collapse of the replication fork, events that activate signaling pathways involved in DDR-mediated cellular senescence (Turinetti et al., 2016). The cell cycle responses involve the activation of Retinoblastoma (Rb) or the p53 protein that subsequently stabilizes specific cyclin-dependent kinase inhibitors, such as p16 and p21, which in turn can trigger senescence (McHugh and Gil, 2018) as illustrated in Figure 2.

MSCs broadly influence the function of various immune cells, including monocytes, macrophages, dendritic cells, T cells, B cells, and natural killer cells, and senescence can alter the immunomodulatory activity of MSCs. The immunomodulatory activity of MSCs is assumed to be an inducible process by the presence of inflammatory cytokines, instead of being a constitutive process. These cells regulate immune responses by releasing soluble factors and via direct cell-contact-dependent mechanisms (Domenis et al., 2018). Ageing can cause elevation in lysosomal activities and dysregulation of cytosolic pH, leading to an increase in the number of senescence-associated beta-

galactosidase (SA-beta-gal)-positive MSCs with suppressed immunomodulatory function (Li et al., 2017a; Lunyak et al., 2017). The immunosuppressive activity of senescent MSCs is reported to be restorable by treatment with CD3/28 and IL-2, restoring the capacity for T cell activation and proliferation (Yu et al., 2014). In the presence of the IFN γ , the immunosuppressive activity of MSCs is enhanced, due to stimulation of the production of inhibitors of inflammation, such as indoleamine-pyrrole 2,3-dioxygenase, Factor H, prostaglandin E2, transforming growth factor beta, and hepatocyte growth factor (Domenis et al., 2018).

Different resistance patterns occur when MSCs are treated with various anti-cancer drugs, many of which are genotoxic. For example, MSCs are relatively sensitive to topoisomerase inhibitors, taxanes, Vinca alkaloids, platinum compounds, and bleomycin. Conversely, they are relatively resistant to cisplatin, etoposide, and vincristine. They show complete resistance to 5-fluorouracil and gemcitabine (Lopez Perez et al., 2019; Münz et al., 2018; Nicolay et al., 2016). BM-MSCs are likewise resistant to antimetabolite chemotherapies such as 5-fluorouracil and gemcitabine. The increased amount of DNA metabolism-related enzymes, such as thymidylate synthase, uridine monophosphate synthase, and thymidine kinase 1, besides the high activity of DDR pathways in MSCs, affects the expression of anti-apoptotic factors, such as Bcl-2 and Bcl-XL, altering the apoptosis process to maintain the stemness of MSCs (Lopez Perez et al., 2019). The p53-mediated DDR can block the stemness properties of the MSCs by promoting the formation of γ H2AX DNA damage foci to induce premature senescence or differentiation of these cells. Knockdown of p53 in MSCs can inhibit the formation of γ H2AX DNA damage foci, suggesting that p53 has a modulatory role in senescence and differentiation (Höfig et al., 2016). In addition, post-senescent MSCs show downregulated expression profiles for DNA-dependent protein kinases in contrast to pre-senescent MSCs which are accompanied with RAD51 protein downregulation after senescence bypass, RAD51 is responsible for specific DNA repair mechanisms. Notably, in contrast to pre- and post-senescence MSCs, tumoral MSCs actively upregulate the expression of DNA repair genes to overcome the excessive DNA damage caused by the higher metabolic rate of cancer cells (Rubio et al., 2008; Tarhriz et al., 2019).

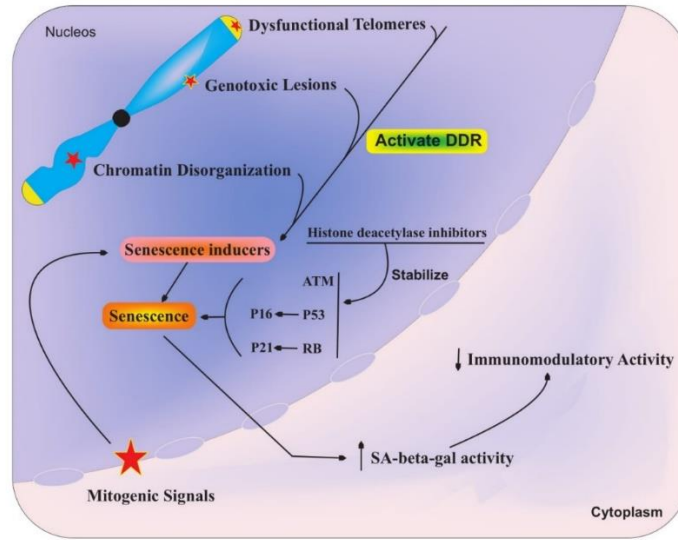


Figure 2. Senescence in MSCs. Different factors can initiate DNA damage response (DDR) in MSCs, including dysfunctional telomeres, genotoxic lesions, and chromatin disorganization, which result in senescence induction. Besides, strong mitogenic signals can induce cellular senescence. Histone deacetylase inhibitors can stabilize senescence-related proteins like ATM, P53, and Retinoblastoma (RB). Senescent cells show decreased immunomodulatory and increased SA-beta-gal activities.

6. DNA-repair mechanisms in MSCs

Various proteins involved in the DDR and DNA repair recognize DNA damage and either restore DNA integrity or induce senescence, differentiation, or apoptosis in MSCs (Krokan and Bjørås, 2013). In all cells, there are different DNA repair pathways that respond based on the DNA damage type. DNA repair activity is under the influence of many modulators, including epigenetics and other factors, which can regulate the gene expression and post-transcriptional modification (ubiquitination, phosphorylation, and acetylation) of proteins involved in DDR pathways (Mens and Ghanbari, 2018; Natarajan, 2016; Patel et al., 2017). Transcriptome analysis of undifferentiated MSCs derived from first-trimester fetal liver tissues indicates a generally higher expression of DNA repair genes in comparison to adult MSCs. Plus, consistent with the discussion earlier (see Section 5), rat MSCs show globally higher expression of DDR genes in comparison to senescent rat MSCs (Frosina, 2010a).

6.1 Base excision repair in MSCs

Base excision repair (BER) is a mechanism to repair DNA base and sugar damage, as well as certain single-strand breaks (SSBs). Base lesions are recognized by DNA glycosylases, such as 8-oxoguanine DNA glycosylase (OGG1), which excise substrate bases from the genome, creating an abasic site intermediate. Abasic sites in DNA are then mainly detected by apurinic/apyrimidinic endodeoxyribonuclease 1 (APEX1) protein, which cleaves the DNA

backbone at the site of the lesion. This protein subsequently collaborates with DNA polymerase beta (POL β), X-ray repair cross-complementing 1 (XRCC1), and a DNA ligase (LIG1 or LIG3) to carry out a BER response. Moreover, APEX1 may also communicate with poly (ADP-Ribose) polymerase 1 (PARP1) at sites of SSB repair (Kennedy et al., 2018). The expression level of specific DNA repair genes, especially the genes encoding BER and inter-strand crosslink-related repair proteins, is significantly upregulated in human embryonic stem cells compared to embryoid bodies (differentiated forms), presumably to maintain genomic integrity (Maynard et al., 2008). Although an enhanced BER capacity is visible in prolonged adipose-derived MSCs, in other types of SCs under similar culture conditions, BER does not appear to be the dominant repair response. For instance, long-term culturing of hESCs can significantly attenuate BER activity through decreased expression of APEX1, a central component of the BER mechanism (Hildrestrand et al., 2009; Krutá et al., 2013).

6.2 Nucleotide excision repair in MSCs

Nucleotide excision repair (NER) responds to DNA adducts that induce helical structural changes (global-genome mechanism) or interfere with transcription processes (transcription-coupled mechanism). NER substrates typically impair transcription and replication processes by distorting the DNA double helix and modifying the DNA structure (Tubbs and Nussenzweig, 2017). Collectively, it is reported that hMSCs show high capacity for NER responses in comparison to other cells (Frosina, 2010b). Moreover, trichothiodystrophy mouse models, which carry homozygous mutations in the NER helicase XPD, show enhanced bone aging and a reduced population of both MSCs and osteoprogenitors cells, because of a decreased proliferation ability (Diderich et al., 2012; Nicolaije et al., 2012). An altered phosphorylation pattern of the ubiquitin-activating enzyme E1 can inhibit the ubiquitination of critical NER proteins, ultimately reducing NER capacity in cells undergoing differentiation (Frosina, 2010c). Thus, NER plays a crucial role in MSC, with stemness and differentiation reducing the activity of this DNA damage response.

6.3 DNA mismatch repair in MSCs

DNA mismatch repair (MMR) removes base mismatches and indels (insertion/deletions of <10 nt) that arise during faulty DNA replication or recombination. MMR activity is significantly correlated with DNA replication and stimulated during S-phase. Although the details of damage-specific recognition are still being worked out, in MMR,

a complex of MSH2 and MSH6 (MutS homologs that make up MUTS α) identify base-base mismatches or small indels, while the MSH2/MSH3 complex (MUTS β) binds to small and large indels. This initial recognition is followed by assembly of MUTL α (a complex of MLH1/PMS2) and Exonuclease 1, in collaboration with replication factor C (RFC), proliferating cell nuclear antigen (PCNA), and POL δ , to facilitate excision of the mispaired nucleotide(s) (Liu et al., 2017). Defective MMR can cause over-duplication and genomic instability of microsatellites in the genome, a hallmark of certain malignancies (Vaish et al., 2005). Mouse models with homozygous mutations in Msh2 or Mlh2 develop stem cell-derived-lymphoid malignancies and many lymphoid tumors with the hallmark of microsatellite instability (Wei et al., 2002). Since MMR deficient cells are not able to stimulate p53-induced G1 cell cycle arrest in response to DNA damage, impaired MMR in SCs can lead to cellular transformation and the generation of cancer stem cells (Vaish, 2007). In addition, Hsa-mir-675 can induce malignant transformation of MSCs by blocking the MMR mechanism. The Hsa-mir-675 upregulates polyubiquitin-binding protein p62 expression and suppresses H3K36me3-hMSH6-SKP2 complex formation, thereby inhibiting the activity of MMR in human cancers (Lu et al., 2019). Interestingly, hsa-mir-1290 plays a similar negative regulatory role in colon cancer cells that exhibit a deficient MMR capacity. Long-term ex-vivo expansion of MSCs can limit MMR activity and alter signaling pathways that regulate the activity of telomerase and the stability of chromosomes in MSCs, eventually resulting in loss of stemness (Jiang et al., 2017).

6.4 Non-homologous end-joining / Homologous recombination

Double strand breaks (DSBs) are known to have extremely deleterious effects on DNA integrity, yet can be repaired by non-homologous end-joining (NHEJ) and homologous recombination (HR) mechanisms (Hare et al., 2016). NHEJ can efficiently join two-ended DSBs through the activities of the end-binding complex, i.e., Ku70, Ku80 and DNA-PK (DNA-dependent protein kinase), in collaboration with end-processing enzymes, such as Artemis, polymerases, XLF, XRCC4 and DNA LIG4, independent of cell cycle status. NHEJ, which often entails some manipulation of the DNA content to reveal microhomologies and facilitate alignment and ligation, is frequently error-prone. Conversely, HR is a mechanism of faithful repair, as it entails the use of an undamaged sister chromatid for accurate resolution of a DSB, but as a result, is restricted to S/G2/M phases of the cell cycle. The detailed mechanism of HR is beyond the scope of this review, but involves 3' to 5' end resection to generate single-stranded DNA stretches needed for strand invasion and the acquisition of sequence information from the sister chromatid (Chapman et al., 2012; Polo and Jackson, 2011). Some of the critical players in HR include the Ser/Thr kinase ataxia telangiectasia mutated (ATM),

the MRN nuclease complex (comprised of MRE11, RAD50 and NBS1), and the homologous pairing protein RAD51, to name a few. Epigenetic modulatory factors, such as DNA methylation and histone acetylation during long-term culture and aging, can regulate NHEJ- and HR-specific gene expression in MSCs. In addition, h-BM-derived MSCs from patients with bone marrow transplantation have elevated DSB repair mechanisms and high ROS-scavenging capacity, making them resistant to infra-red exposure that is dependent on DNA repair (Frosina, 2010a). hMSC are in fact broadly resistant to infra-red exposure and exhibit higher telomerase activity and both SSB and DSB repair capacity in comparison to other cells (Frosina, 2010a).

6.5 Regulation of DNA repair pathways through miRNAs in MSCs

The asymmetric cell division of MSCs is a complicated process that is regulated by different transcription factors, epigenetic modulators, and hormones. The role of miRNAs in the regulation of proliferation and cell cycle is well-established in MSCs by several studies. For example, has-miR-16 efficiently targets mRNAs of cyclin E in MSCs to induce cell cycle arrest and inhibit proliferation and the DDR. Moreover, this miRNA downregulates the expression of BMI1, a proto-oncogene, and other poly-comb proteins, including Ring Finger Protein 1 (RING1A), RING1B, and Enhancer of Zeste 2 Polycomb Repressive Complex 2 Subunit (EZH2). In addition, has-miR-16 alters the expression level of proteins that are involved in BMI1-dependent ubiquitination pathways (Mens and Ghanbari, 2018; Patel et al., 2017). Chaudhry et al. found that elevated expression of has-miR-16 is associated with reduction in DNA-PK expression and activity (Chaudhry et al., 2010). Also, has-miR-143 can suppress ERK5 gene expression (MAPK's family member) and downregulate the expression of cyclin D and CDK6 genes, resulting in the activation of the S phase checkpoints. DNA damage upregulates has-miR-143 expression via p53 and MDM2 proto-oncogenes. The established positive feedback supports the function of p53 in facilitating DNA damage-mediated cell death (Mens and Ghanbari, 2018; Zhang et al., 2013). Upregulation of has-miR-27 promotes osteogenic differentiation of MSCs through targeting of APC gene expression, which modifies the G2/M transition. Also, downregulation of has-miR-27 leads to overexpression of set of genes including ERK1/2, ERK5, TGF- β 1 and KLF5 that results in adipocyte differentiation of the MSCs (Mens and Ghanbari, 2018). Moreover, has-miR-27a can modify the DDR in γ -radiated lung cancer A549 cells by downregulation of the ATM gene and modulation of DSB rejoining kinetics following irradiation damage (Di Francesco et al., 2013a). ATM controls the cell cycle checkpoints (G1-S, S, and G2-M), and

downregulation of this gene by has-miR-27 adversely affects the p53-p21 axis and results in impaired cell cycle arrest and DDR following radiation exposure (Di Francesco et al., 2013b).

6.6 Telomeres in DDR of MSCs

A lack of telomeric elements at the end of chromosomes can result in DNA degradation and genomic instability in the form of end to end fusion. In addition, telomere defects can activate DNA damage checkpoints and the DDR (Blackburn, 2000). Nucleotide gaps, oxidized bases, abasic sites, and uncorrected nucleotides can alter DNA sequences by inhibiting replication fork activity, potentially causing loss of telomere structure integrity (Opresko et al., 2005; von Zglinicki, 2002). Telomere length shortening is associated with DNA damage, and the accumulation of prelamin A, a nuclear envelope protein precursor, can activate DNA damage-related mechanisms in hMSCs (Infante et al., 2014). The DDR is characterized by ATM activation and the formation of DNA-damage foci containing p53BP1 and γ H2A.X proteins around DSB sites (Borodkina et al., 2014). Hyper phosphorylation of histone H2A at serine 139 is a marker of DNA DSBs in old BM-MSCs (Asumda and Chase, 2011b; Prendergast et al., 2011).

Several studies have shown that SIRT1 and XRCC5 (a.k.a., KU80) play an essential role in cellular stress responses that are associated with telomere biology and DNA damage checkpoint regulation. XRCC5 is involved in NHEJ DSB repair, and SIRT1 can negatively regulate p53 activity and inhibit growth arrest, apoptosis, and senescence (Brandl et al., 2011; Thacker and Zdzienicka, 2004, 2003). Telomere uncapping, DNA damage foci formation at telomere sites, and oxidative insults can induce cellular senescence through activation of the p21-p53 axis (Ben-Porath and Weinberg, 2005; Brandl et al., 2011; Herbig et al., 2004).

6.7 Induction of DDR in MSCs

Ultraviolet radiation, osmotic shock, hypoxia, pro-inflammatory cytokines, and oxidative stress are the main extrinsic stressors that can activate the p38 MAPK pathway, a process involved in differentiation, apoptosis and autophagy. ROS mainly induce the loss of differentiation capacity of MSCs rather than affecting their proliferation ability. Continued exposure of MSCs to ROS can induce senescence; however, a high amount of ROS has cytotoxic effects and promotes mutagenic damages in healthy cells. Conversely, a low concentration of ROS in MSCs is essential to maintain cell proliferation, self-renewal ability, regulation of differentiation, and intracellular signaling (Yang et al.,

2015). Serum extracted from aged animals can be used to induce high levels of ROS in MSCs, through a mechanism that likely involves changing the expression pattern of proteins related to mitochondria, unfolded protein binding and stress responses; such serum can also increase intracellular ROS production and drive the accumulation of oxidatively damaged proteins (Geißler et al., 2013). This high level of ROS can activate the DDR and Wnt/ β -catenin signaling through the p53/p21 pathway, ultimately promoting aging in MSCs (Zhang et al., 2011). Physical stressors, such as ionizing and infrared radiation, as well as starvation, can significantly reduce the cell growth rate and induce early senescence in MSCs. Nevertheless, irradiated and starved MSCs conserve their immune-phenotype, maintain differentiation ability into adipocytes or osteoblasts, and prevent mitogen-induced T-cell proliferation. These cells do not show any significant changes on the genomic level (e.g., DNA mutation); however, supramaximal stresses can stimulate ROS production in these cells, activating the DDR through ATM. Moreover, NHEJ-related genes, including PRKDC (a.k.a., DNA-PK), RAD50, and H2A histone family member X (H2AFX), as well as HR-related genes, including ATM, RPA1, BRCA1, and RAD51, are upregulated in MSCs in response to external stresses. Overall, MSCs prefer to maintain their stemness under stress conditions rather than acquire malignant features (Alessio et al., 2017, 2015; Conforti et al., 2016). Irradiated mouse MSCs can preserve proliferation and differentiation capacity for the long-term through H2AX overexpression, S and G2/M DNA damage checkpoint activation, and effective repair of infrared-induced DSBs. These cells also increase survival through the upregulation of DDR factors (e.g., ATM, Chk2, and LIG4) and anti-apoptotic genes (e.g., Bcl-2 and Bcl-XL) and the downregulation of pro-apoptotic factors (e.g., Bim and Puma) (Sugrue et al., 2013). Cisplatin can induce the DDR in MSCs, activating the p53-p21 axis to arrest the cell cycle. MSCs derived from healthy individuals and chronic lymphocytic leukemia patients exhibit different responses to treatment with cisplatin, fludarabine, cyclophosphamide, and rituximab. The MSCs derived from treated patients show a decreased expansion capacity in comparison to normal MSCs in response to the cytotoxic effects of DNA-damaging agents (Prendergast et al., 2011).

Conclusion

MSCs can modulate tissue homeostasis. Also, they can control tissue repair and regeneration capacity in aging-associated degenerative diseases. Although MSCs are located in hypoxic niches to keep away from oxidative stressors and maintain their stemness properties, they are still susceptible to intrinsic and extrinsic DNA-damaging agents. The

primary responses of MSCs to DNA damage are the production of a considerable amount of anti-oxidants and activation of the DDR to reduce genotoxicity.

However, irreparable damage to DNA in MSCs can activate differentiation, senescence, or regulated cell death processes. Moreover, DNA damage can reduce the stemness, proliferation, and differentiation properties of MSCs, thereby decreasing tissue repair and regeneration capacity. Understanding the DNA damage repair mechanisms in MSCs can widen our knowledge about the potential clinical and therapeutic applications of these cells. Furthermore, the unraveling of the DDR mechanisms of MSCs will allow us to improve the strategies of MSC use in in-vitro and ex-vivo approaches. A comprehensive understanding will ultimately provide critical insight into the possible role of MSCs in the treatment of age-related diseases during biological aging.

Declaration of Competing Interests

The authors have no conflict of interest to declare relevant to the content of this review.

Acknowledgment

There were no funding resources for the current study.

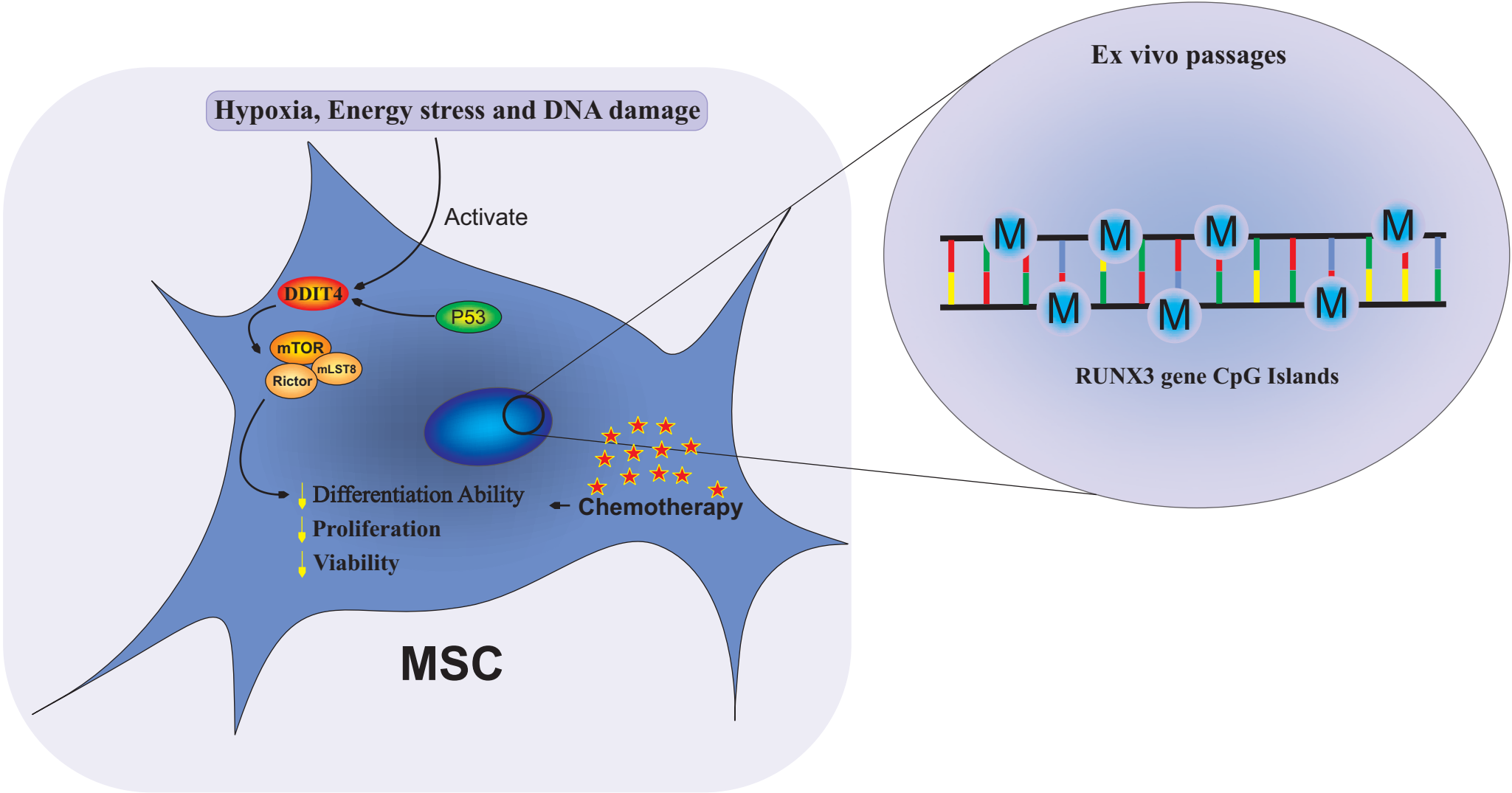
Reference

- Alessio, N., Capasso, S., Di Bernardo, G., Cappabianca, S., Casale, F., Calarco, A., Cipollaro, M., Peluso, G., Galderisi, U., 2017. Mesenchymal stromal cells having inactivated RB1 survive following low irradiation and accumulate damaged DNA: Hints for side effects following radiotherapy. *Cell cycle* (Georgetown, Tex.) 16, 251–258. <https://doi.org/10.1080/15384101.2016.1175798>
- Alessio, N., Del Gaudio, S., Capasso, S., Di Bernardo, G., Cappabianca, S., Cipollaro, M., Peluso, G., Galderisi, U., 2015. Low dose radiation induced senescence of human mesenchymal stromal cells and impaired the autophagy process. *Oncotarget* 6, 8155–66. <https://doi.org/10.18632/oncotarget.2692>
- Alves, H., Munoz-Najar, U., De Wit, J., Renard, A.J.S., Hoeijmakers, J.H.J., Sedivy, J.M., Van Blitterswijk, C., De Boer, J., 2010. A link between the accumulation of DNA damage and loss of multi-potency of human mesenchymal stromal cells. *Journal of Cellular and Molecular Medicine* 14, 2729–2738. <https://doi.org/10.1111/j.1582-4934.2009.00931.x>
- Ames, B.N., Profet, M., Gold, L.S., 1990. Dietary pesticides (99.99% all natural). *Proceedings of the National Academy of Sciences of the United States of America* 87, 7777–81.
- Aqmasheh, S., Shamsasanjan, K., Akbarzadehlaleh, P., Pashoutan Sarvar, D., Timari, H., 2017. Effects of Mesenchymal Stem Cell Derivatives on Hematopoiesis and Hematopoietic Stem Cells. *Advanced pharmaceutical bulletin* 7, 165–177. <https://doi.org/10.15171/apb.2017.021>
- Asumda, F.Z., Chase, P.B., 2011a. Age-related changes in rat bone-marrow mesenchymal stem cell plasticity. *BMC Cell Biology* 12, 44. <https://doi.org/10.1186/1471-2121-12-44>
- Asumda, F.Z., Chase, P.B., 2011b. Age-related changes in rat bone-marrow mesenchymal stem cell plasticity. *BMC cell biology* 12, 44.
- Ben-Porath, I., Weinberg, R.A., 2005. The signals and pathways activating cellular senescence. *The international journal of biochemistry & cell biology* 37, 961–976.
- Benameur, L., Charif, N., Li, Y., Stoltz, J.-F., de Isla, N., 2015. Toward an understanding of mechanism of aging-induced oxidative stress in human mesenchymal stem cells. *Bio-medical materials and engineering* 25, 41–6. <https://doi.org/10.3233/BME-141247>
- Bennett, J.W., Klich, M., 2003. Mycotoxins. *Clinical microbiology reviews* 16, 497–516.
- Bielak-Zmijewska, A., Mosieniak, G., Sikora, E., 2018. Is DNA damage indispensable for stress-induced senescence? *Mechanisms of Ageing and Development* 170, 13–21. <https://doi.org/10.1016/j.mad.2017.08.004>
- Blackburn, E.H., 2000. Telomere states and cell fates. *Nature* 408, 53.
- Bork, S., Pfister, S., Witt, H., Horn, P., Korn, B., Ho, A.D., Wagner, W., 2010. DNA methylation pattern changes upon long-term culture and aging of human mesenchymal stromal cells. *Aging Cell* 9, 54–63. <https://doi.org/10.1111/j.1474-9726.2009.00535.x>
- Borodkina, A. V., Shatrova, A.N., Pugovkina, N.A., Zemelko, V.I., Nikolsky, N.N., Burova, E.B., 2014. Different protective mechanisms of human embryonic and endometrium-derived mesenchymal stem cells under oxidative stress. *Cell and Tissue Biology* 8, 11–21. <https://doi.org/10.1134/S1990519X14010040>
- Brandl, A., Meyer, M., Bechmann, V., Nerlich, M., Angele, P., 2011. Oxidative stress induces senescence in human mesenchymal stem cells. *Experimental cell research* 317, 1541–1547.
- Broekman, W., Amatgahim, G.D., de Mooij-Eijk, Y., Oostendorp, J., Roelofs, H., Taube, C., Stolk, J., Hiemstra, P.S., 2016. TNF- α and IL-1 β -activated human mesenchymal stromal cells increase airway epithelial wound healing in vitro via activation of the epidermal growth factor receptor. *Respiratory Research* 17, 3. <https://doi.org/10.1186/s12931-015-0316-1>
- Caplan, A., 2009. Why are MSCs therapeutic? New data: new insight. *The Journal of Pathology* 217, 318–324. <https://doi.org/10.1002/path.2469>
- Chapman, J.R., Taylor, M.R.G., Boulton, S.J., 2012. Playing the end game: DNA double-strand break repair pathway choice. *Molecular cell* 47, 497–510.
- Chatterjee, N., Walker, G.C., 2017. Mechanisms of DNA damage, repair, and mutagenesis. *Environmental and molecular mutagenesis* 58, 235–263. <https://doi.org/10.1016/j.jmrras.2015.03.003>
- Chaudhry, M.A., Sachdeva, H., Omaruddin, R.A., 2010. Radiation-Induced Micro-RNA Modulation in Glioblastoma Cells Differing in DNA-Repair Pathways. *DNA and Cell Biology* 29, 553–561. <https://doi.org/10.1089/dna.2009.0978>
- Conforti, A., Starc, N., Biagini, S., Tomao, L., Pitisci, A., Algeri, M., Sirleto, P., Novelli, A., Grisendi, G., Candini, O., Carella, C., Dominici, M., Locatelli, F., Bernardo, M.E., 2016. Resistance to neoplastic transformation of ex-vivo expanded human mesenchymal stromal cells after exposure to supramaximal physical and chemical stress. *Oncotarget* 7, 77416–77429. <https://doi.org/10.18632/oncotarget.12678>
- Cruet-Hennequart, S., Prendergast, A.M., Shaw, G., Barry, F.P., Carty, M.P., 2012. Doxorubicin induces the DNA damage response in cultured human mesenchymal stem cells. *International Journal of Hematology* 96, 649–656. <https://doi.org/10.1007/s12185-012-1196-5>
- Desouky, O., Ding, N., Zhou, G., 2015. Targeted and non-targeted effects of ionizing radiation. *Journal of Radiation Research and Applied Sciences* 8, 247–254. <https://doi.org/10.1016/j.jmrras.2015.03.003>
- Di Francesco, A., De Pittà, C., Moret, F., Barbieri, V., Celotti, L., Mognato, M., 2013a. The DNA-Damage Response to γ -Radiation Is Affected by miR-27a in A549 Cells. *International Journal of Molecular Sciences* 14, 17881–17896. <https://doi.org/10.3390/ijms140917881>
- Di Francesco, A., De Pittà, C., Moret, F., Barbieri, V., Celotti, L., Mognato, M., 2013b. The DNA-Damage Response to γ -radiation is affected by miR-27a in A549 cells. *International Journal of Molecular Sciences* 14, 17881–17896. <https://doi.org/10.3390/ijms140917881>
- Diderich, K.E.M., Nicolaije, C., Priemel, M., Waarsing, J.H., Day, J.S., Brandt, R.M.C., Schilling, A.F., Botter, S.M., Weinans, H., van der Horst, G.T.J., Hoeijmakers, J.H.J., van Leeuwen, J.P.T.M., 2012. Bone fragility and decline in stem cells in prematurely aging DNA repair deficient trichothiodystrophy mice. *AGE* 34, 845–861. <https://doi.org/10.1007/s11357-011-9291-8>
- Domenis, R., Cifù, A., Quaglia, S., Pistis, C., Moretti, M., Vicario, A., Parodi, P.C., Fabris, M., Niazi, K.R., Soon-Shiong, P., Curcio, F., 2018. Pro inflammatory stimuli enhance the immunosuppressive functions of adipose mesenchymal stem cells-derived exosomes. *Scientific Reports* 8, 1–11. <https://doi.org/10.1038/s41598-018-31707-9>
- Ejtehadifar, M., Shamsasanjan, K., Akbarzadehlaleh, P., Zahedi, S., Kazemi, N., 2016. The Effects of Hypoxia on U937 Cell Line in Mesenchymal Stem Cells Co-Culture System. *Advanced pharmaceutical bulletin* 6, 645–650. <https://doi.org/10.15171/apb.2016.079>
- Eleuteri, S., Fierabracci, A., 2019. Insights into the secretome of mesenchymal stem cells and its potential applications. *International Journal of Molecular Sciences*. <https://doi.org/10.3390/ijms20184597>
- Ellisen, L.W., Ramsayer, K.D., Johannessen, C.M., Yang, A., Beppu, H., Minda, K., Oliner, J.D., McKeon, F., Haber, D.A., 2002. REDD1, a developmentally regulated transcriptional target of p63 and p53, links p63 to regulation of reactive oxygen species. *Molecular cell* 10, 995–1005. [https://doi.org/10.1016/S1097-2765\(02\)00706-2](https://doi.org/10.1016/S1097-2765(02)00706-2)
- Ferreira, J.R., Teixeira, G.Q., Santos, S.G., Barbosa, M.A., Almeida-Porada, G., Gonçalves, R.M., 2018. Mesenchymal Stromal Cell Secretome: Influencing Therapeutic Potential by Cellular Pre-conditioning. *Frontiers in Immunology* 9, 2837. <https://doi.org/10.3389/FIMMU.2018.02837>
- Fitzsimmons, R.E.B., Mazurek, M.S., Soos, A., Simmons, C.A., 2018. Mesenchymal Stromal/Stem Cells in Regenerative Medicine and Tissue Engineering. *Stem Cells International* 2018, 1–16. <https://doi.org/10.1155/2018/8031718>
- Frosina, G., 2010a. The bright and the dark sides of DNA repair in stem cells. *BioMed Research International* 2010.
- Frosina, G., 2010b. The bright and the dark sides of DNA repair in stem cells. *Journal of biomedicine & biotechnology* 2010, 845396. <https://doi.org/10.1155/2010/845396>
- Frosina, G., 2010c. The bright and the dark sides of DNA repair in stem cells. *Journal of biomedicine & biotechnology* 2010, 845396. <https://doi.org/10.1155/2010/845396>
- Gafer-Gvili, A., Zingerman, B., Rozen-Zvi, B., Ori, Y., Green, H., Lubin, I., Malachi, T., Gafer, U., Herman-Edelstein, M., 2013. Oxidative Stress-Induced DNA Damage and Repair in Human Peripheral Blood Mononuclear Cells: Protective Role of Hemoglobin. *PLoS ONE* 8, e68341. <https://doi.org/10.1371/journal.pone.0068341>
- Geißler, S., Textor, M., Schmidt-Bleek, K., Klein, O., Thiele, M., Ellinghaus, A., Jacobi, D., Ode, A., Perka, C., Dienelt, A., Klose, J., Kasper, G., Duda, G.N., Strube, P., 2013. In serum veritas-in serum sanitas? Cell non-autonomous aging compromises differentiation and survival of mesenchymal stromal cells via the oxidative stress pathway. *Cell Death and Disease* 4, e970. <https://doi.org/10.1038/cddis.2013.501>

- Gharibi, B., Ghuman, M., Hughes, F.J., 2016. DDIT4 regulates mesenchymal stem cell fate by mediating between HIF1 α and mTOR signalling. *Scientific Reports* 6, 36889. <https://doi.org/10.1038/srep36889>
- Hare, L., Gencheva, M., Evans, R., Fortney, J., Piktet, D., Vos, J.A., Howell, D., Gibson, L.F., 2016. *In Vitro* Expansion of Bone Marrow Derived Mesenchymal Stem Cells Alters DNA Double Strand Break Repair of Etoposide Induced DNA Damage. *Stem Cells International* 2016, 1–11. <https://doi.org/10.1155/2016/8270464>
- Harvey, R., 1991. Polycyclic aromatic hydrocarbons: chemistry and carcinogenicity.
- He, S., Sharpless, N.E., 2017. Senescence in Health and Disease. *Cell* 169, 1000–1011. <https://doi.org/10.1016/J.CELL.2017.05.015>
- Henle, E.S., Linn, S., 1997. Formation, prevention, and repair of DNA damage by iron/hydrogen peroxide. *The Journal of biological chemistry* 272, 19095–8. <https://doi.org/10.1074/JBC.272.31.19095>
- Herbig, U., Jobling, W.A., Chen, B.P.C., Chen, D.J., Sedivy, J.M., 2004. Telomere shortening triggers senescence of human cells through a pathway involving ATM, p53, and p21(CIP1), but not p16(INK4a). *Molecular cell* 14, 501–13.
- Hildrestrand, G.A., Neurauder, C.G., Diep, D.B., Castellanos, C.G., Krauss, S., Bjoras, M., Luna, L., 2009. Expression patterns of Neil3 during embryonic brain development and neoplasia. *BMC Neuroscience* 10, 45. <https://doi.org/10.1186/1471-2202-10-45>
- Höfig, I., Ingawale, Y., Atkinson, M.J., Hertlein, H., Nelson, P.J., Rosemann, M., 2016. p53-Dependent Senescence in Mesenchymal Stem Cells under Chronic Normoxia Is Potentiated by Low-Dose γ -Irradiation. *Stem Cells International* 2016, 1–11. <https://doi.org/10.1155/2016/6429853>
- Hou, J., Han, Z., Jing, Y., Yang, X., Zhang, S., Sun, K., Hao, C., Meng, Y., Yu, F., Liu, X., Shi, Y., Wu, M., Zhang, L., Wei, L., 2013. Autophagy prevents irradiation injury and maintains stemness through decreasing ROS generation in mesenchymal stem cells. *Cell death & disease* 4, e844. <https://doi.org/10.1038/cddis.2013.338>
- Infante, A., Gago, A., de Eguino, G.R., Calvo-Fernández, T., Gómez-Vallejo, V., Llop, J., Schlangen, K., Fullaondo, A., Aransay, A.M., Martín, A., 2014. Prelamin A accumulation and stress conditions induce impaired Oct-1 activity and autophagy in prematurely aged human mesenchymal stem cell. *Aging (Albany NY)* 6, 264.
- Jiang, T., Xu, G., Wang, Q., Yang, L., Zheng, L., Zhao, J., Zhang, X., 2017. In vitro expansion impaired the stemness of early passage mesenchymal stem cells for treatment of cartilage defects. *Cell Death & Disease* 8, e2851–e2851. <https://doi.org/10.1038/cddis.2017.215>
- Kennedy, E.E., Caffrey, P.J., Delaney, S., 2018. Initiating base excision repair in chromatin. *DNA Repair* 71, 87–92. <https://doi.org/10.1016/j.dnarep.2018.08.011>
- Kiefer, J., 2007. Effects of Ultraviolet Radiation on DNA, in: *Chromosomal Alterations*. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 39–53. https://doi.org/10.1007/978-3-540-71414-9_3
- Krokan, H.E., Björås, M., 2013. Base excision repair. *Cold Spring Harbor perspectives in biology* 5, a012583. <https://doi.org/10.1101/cshperspect.a012583>
- Krutá, M., Bálek, L., Hejnová, R., Dobšáková, Z., Eiselleová, L., Matulka, K., Bárta, T., Fojtik, P., Fajkus, J., Hampl, A., Dvořák, P., Rotrekl, V., 2013. Decrease in Abundance of Apurinic/Pyrimidinic Endonuclease Causes Failure of Base Excision Repair in Culture-Adapted Human Embryonic Stem Cells. *STEM CELLS* 31, 693–702. <https://doi.org/10.1002/stem.1312>
- Li, Y., Wu, Q., Wang, Y., Li, L., Bu, H., Bao, J., 2017a. Senescence of mesenchymal stem cells (Review). *International Journal of Molecular Medicine* 39, 775–782. <https://doi.org/10.3892/ijmm.2017.2912>
- Li, Y., Wu, Q., Wang, Y., Li, L., Bu, H., Bao, J., 2017b. Senescence of mesenchymal stem cells (Review). *International Journal of Molecular Medicine* 39, 775–782. <https://doi.org/10.3892/ijmm.2017.2912>
- Lindahl, T., Barnes, D.E., 2000. Repair of endogenous DNA damage. *Cold Spring Harbor symposia on quantitative biology* 65, 127–33.
- Liu, D., Keijzers, G., Rasmussen, L.J., 2017. DNA mismatch repair and its many roles in eukaryotic cells. *Mutation Research/Reviews in Mutation Research* 773, 174–187. <https://doi.org/10.1016/J.MRREV.2017.07.001>
- Loeb, L.A., Monnat, R.J., 2008. DNA polymerases and human disease. *Nature Reviews Genetics* 9, 594–604. <https://doi.org/10.1038/nrg2345>
- Lombard, D.B., Chua, K.F., Mostoslavsky, R., Franco, S., Gostissa, M., Alt, F.W., 2005. DNA repair, genome stability, and aging. *Cell* 120, 497–512.
- Lopez Perez, R., Münz, F., Vidoni, D., Rühle, A., Trinh, T., Sisombath, S., Zou, B., Wuchter, P., Debus, J., Grosu, A.-L., Saffrich, R., Huber, P.E., Nicolay, N.H., 2019. Mesenchymal stem cells preserve their stem cell traits after exposure to antimetabolite chemotherapy. *Stem Cell Research* 40, 101536. <https://doi.org/10.1016/J.SCR.2019.101536>
- Lu, Y., Song, S., Jiang, X., Meng, Q., Wang, C., Li, X., Yang, Y., Xin, X., Zheng, Q., Wang, L., Pu, H., Gui, X., Li, T., Lu, D., 2019. miR675 Accelerates Malignant Transformation of Mesenchymal Stem Cells by Blocking DNA Mismatch Repair. *Molecular therapy. Nucleic acids* 14, 171–183. <https://doi.org/10.1016/j.omtn.2018.11.010>
- Lunyak, V. V., Amaro-Ortiz, A., Gaur, M., 2017. Mesenchymal stem cells secretory responses: Senescence messaging secretome and immunomodulation perspective. *Frontiers in Genetics*. <https://doi.org/10.3389/fgene.2017.00220>
- Luoto, K.R., Kumareswaran, R., Bristow, R.G., 2013. Tumor hypoxia as a driving force in genetic instability. *Genome Integrity* 4, 5. <https://doi.org/10.1186/2041-9414-4-5>
- Mani, C., Reddy, P.H., Palle, K., 2019. DNA repair fidelity in stem cell maintenance, health, and disease. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*. <https://doi.org/10.1016/J.BBADS.2019.03.017>
- Maynard, S., Swistowska, A.M., Lee, J.W., Liu, Y., Liu, S.-T., Da Cruz, A.B., Rao, M., de Souza-Pinto, N.C., Zeng, X., Bohr, V.A., 2008. Human Embryonic Stem Cells Have Enhanced Repair of Multiple Forms of DNA Damage. *Stem Cells* 26, 2266–2274. <https://doi.org/10.1634/stemcells.2007-1041>
- McGhee, N.K., Jefferson, L.S., Kimball, S.R., 2009. Elevated Corticosterone Associated with Food Deprivation Upregulates Expression in Rat Skeletal Muscle of the mTORC1 Repressor, REDD1. *The Journal of Nutrition* 139, 828–834. <https://doi.org/10.3945/jn.108.099846>
- McHugh, D., Gil, J., 2018. Senescence and aging: Causes, consequences, and therapeutic avenues. *The Journal of cell biology* 217, 65–77. <https://doi.org/10.1083/jcb.201708092>
- Mens, M.M.J., Ghanbari, M., 2018. Cell Cycle Regulation of Stem Cells by MicroRNAs. *Stem Cell Reviews and Reports* 14, 309–322. <https://doi.org/10.1007/s12015-018-9808-y>
- Moravej, A., Kouhpayeh, A., Geramizadeh, B., Azarpira, N., Yaghoobi, R., Mansoori, Y., Karimi, M.-H., 2019. The Effect of Mesenchymal Stem Cells on the Expression of IDO and Qa2 Molecules in Dendritic Cells. *Advanced pharmaceutical bulletin* 9, 56–63. <https://doi.org/10.15171/apb.2019.007>
- Munro, J., Barr, N.I., Ireland, H., Morrison, V., Parkinson, E.K., 2004. Histone deacetylase inhibitors induce a senescence-like state in human cells by a p16-dependent mechanism that is independent of a mitotic clock. *Experimental Cell Research* 295, 525–538. <https://doi.org/10.1016/j.yexcr.2004.01.017>
- Münz, F., Lopez Perez, R., Trinh, T., Sisombath, S., Weber, K.-J., Wuchter, P., Debus, J., Saffrich, R., Huber, P.E., Nicolay, N.H., 2018. Human mesenchymal stem cells lose their functional properties after paclitaxel treatment. *Scientific Reports* 8, 312. <https://doi.org/10.1038/s41598-017-18862-1>
- Natarajan, V., 2016. Regulation of DNA repair by non-coding miRNAs. *Non-coding RNA Research* 1, 64–68. <https://doi.org/10.1016/J.NCRNA.2016.10.002>
- Neutelings, T., Lambert, C.A., Nussgens, B. V., Colige, A.C., 2013. Effects of Mild Cold Shock (25°C) Followed by Warming Up at 37°C on the Cellular Stress Response. *PLoS ONE* 8, e69687. <https://doi.org/10.1371/journal.pone.0069687>
- Nicolaije, C., Diderich, K.E.M., Botter, S.M., Priemel, M., Waarsing, J.H., Day, J.S., Brandt, R.M.C., Schilling, A.F., Weinans, H., Van der Eerden, B.C., van der Horst, G.T.J., Hoesjmakers, J.H.J., van Leeuwen, J.P.T.M., 2012. Age-Related Skeletal Dynamics and Decrease in Bone Strength in DNA Repair Deficient Male Trichothiodystrophy Mice. *PLoS ONE* 7, e35246. <https://doi.org/10.1371/journal.pone.0035246>
- Nicolay, N.H., Liang, Y., Lopez Perez, R., Bostel, T., Trinh, T., Sisombath, S., Weber, K.-J., Ho, A.D., Debus, J., Saffrich, R., Huber, P.E., 2015. Mesenchymal stem cells are resistant to carbon ion radiotherapy. *Oncotarget* 6, 2076–87. <https://doi.org/10.18632/oncotarget.2857>
- Nicolay, N.H., Rühle, A., Perez, R.L., Trinh, T., Sisombath, S., Weber, K.-J., Schmezer, P., Ho, A.D., Debus, J., Saffrich, R., Huber, P.E., 2016. Mesenchymal stem cells exhibit resistance to topoisomerase inhibition. *Cancer Letters* 374, 75–84. <https://doi.org/10.1016/j.canlet.2016.02.007>
- O'Driscoll, M., Macpherson, P., Xu, Y.Z., Karan, P., 1999. The cytotoxicity of DNA carboxymethylation and methylation by the model carboxymethylating agent azaserine in human cells. *Carcinogenesis* 20, 1855–62.
- Ogryzko, V. V., Hirai, T.H., Russanova, V.R., Barbie, D.A., Howard, B.H., 1996. Human fibroblast commitment to a senescence-like state in response to histone deacetylase inhibitors is cell cycle dependent. *Molecular and cellular biology* 16, 5210–8.
- Opreško, P.L., Fan, J., Danzy, S., Wilson III, D.M., Bohr, V.A., 2005. Oxidative damage in telomeric DNA disrupts recognition by TRF1 and TRF2. *Nucleic acids*

- research 33, 1230–1239.
- Osakabe, A., Tachiwana, H., Kagawa, W., Horikoshi, N., Matsumoto, S., Hasegawa, M., Matsumoto, N., Toga, T., Yamamoto, J., Hanaoka, F., Thomä, N.H., Sugawara, K., Iwai, S., Kurumizaka, H., 2015. Structural basis of pyrimidine-pyrimidone (6–4) photoproduct recognition by UV-DDB in the nucleosome. *Scientific Reports* 5, 16330. <https://doi.org/10.1038/srep16330>
- Pashoutan Sarvar, D., Karimi, M.H., Movassaghpour, A., Akbarzadehlaleh, P., Aqmasheh, S., Timari, H., Shamsasenjan, K., 2018. The Effect of Mesenchymal Stem Cell-Derived Microvesicles on Erythroid Differentiation of Umbilical Cord Blood-Derived CD34+ Cells. *Advanced pharmaceutical bulletin* 8, 291–296. <https://doi.org/10.15171/apb.2018.034>
- Patel, N., Garikapati, K.R., Pandita, R.K., Singh, D.K., Pandita, T.K., Bhadra, U., Bhadra, M.P., 2017. miR-15a/miR-16 down-regulates BMI1, impacting Ub-H2A mediated DNA repair and breast cancer cell sensitivity to doxorubicin. *Scientific Reports* 7, 4263. <https://doi.org/10.1038/s41598-017-02800-2>
- Polo, S.E., Jackson, S.P., 2011. Dynamics of DNA damage response proteins at DNA breaks: a focus on protein modifications. *Genes & development* 25, 409–433.
- Pommier, Y., Barcelo, J.M., Rao, V.A., Sordet, O., Jobson, A.G., Thibaut, L., Miao, Z., Seiler, J.A., Zhang, H., Marchand, C., Agama, K., Nitiss, J.L., Redon, C., 2006. Repair of Topoisomerase I-Mediated DNA Damage, in: *Progress in Nucleic Acid Research and Molecular Biology*. pp. 179–229. [https://doi.org/10.1016/S0079-6603\(06\)81005-6](https://doi.org/10.1016/S0079-6603(06)81005-6)
- Prendergast, A.M., Cruet-Hennequart, S., Shaw, G., Barry, F.P., Carty, M.P., 2011. Activation of DNA damage response pathways in human mesenchymal stem cells exposed to cisplatin or γ -irradiation. *Cell cycle* 10, 3768–3777.
- Roos, W.P., Kaina, B., 2006. DNA damage-induced cell death by apoptosis. *Trends in molecular medicine* 12, 440–450.
- Rubio, D., Garcia, S., Paz, M.F., De la Cueva, T., Lopez-Fernandez, L.A., Lloyd, A.C., Garcia-Castro, J., Bernad, A., 2008. Molecular Characterization of Spontaneous Mesenchymal Stem Cell Transformation. *PLoS ONE* 3, e1398. <https://doi.org/10.1371/journal.pone.0001398>
- Skipper, P.L., Kim, M.Y., Sun, H.-L.P., Wogan, G.N., Tannenbaum, S.R., 2010. Monocyclic aromatic amines as potential human carcinogens: old is new again. *Carcinogenesis* 31, 50–58. <https://doi.org/10.1093/carcin/bgp267>
- Srinivasan, A., Fults, M.L., Supronowicz, P., Esquivel, R., Zamilpa, R., 2019. Mesenchymal Stem Cell-Derived Products for Tissue Repair and Regeneration, in: *A Roadmap to Non-Hematopoietic Stem Cell-Based Therapeutics*. Elsevier, pp. 419–441. <https://doi.org/10.1016/b978-0-12-811920-4.00017-3>
- Sugimura, T., 1986. Past, present, and future of mutagens in cooked foods. *Environmental Health Perspectives* 67, 5–10. <https://doi.org/10.1289/ehp.86675>
- Sugrue, T., Brown, J.A.L., Lowndes, N.F., Ceredig, R., 2013. Multiple Facets of the DNA Damage Response Contribute to the Radioresistance of Mouse Mesenchymal Stromal Cell Lines. *STEM CELLS* 31, 137–145. <https://doi.org/10.1002/stem.1222>
- Tarhriz, V., Bandehpour, M., Dastmalchi, S., Ouladsahebmadarek, E., Zarredar, H., Eyvazi, S., 2019. Overview of CD24 as a new molecular marker in ovarian cancer. *Journal of Cellular Physiology* 234, 2134–2142. <https://doi.org/10.1002/jcp.27581>
- Thacker, J., Zdzenicka, M.Z., 2004. The XRCC genes: expanding roles in DNA double-strand break repair. *DNA repair* 3, 1081–1090.
- Thacker, J., Zdzenicka, M.Z., 2003. The mammalian XRCC genes: their roles in DNA repair and genetic stability. *DNA repair* 2, 655–672.
- Timari, H., Shamsasenjan, K., Movassaghpour, A., Akbarzadehlaleh, P., Pashoutan Sarvar, D., Aqmasheh, S., 2017. The Effect of Mesenchymal Stem Cell-Derived Extracellular Vesicles on Hematopoietic Stem Cells Fate. *Advanced pharmaceutical bulletin* 7, 531–546. <https://doi.org/10.15171/apb.2017.065>
- Tubbs, A., Nussenzweig, A., 2017. Endogenous DNA Damage as a Source of Genomic Instability in Cancer. *Cell* 168, 644–656. <https://doi.org/10.1016/j.cell.2017.01.002>
- Turinetto, V., Vitale, E., Giachino, C., 2016. Senescence in Human Mesenchymal Stem Cells: Functional Changes and Implications in Stem Cell-Based Therapy. *International journal of molecular sciences* 17. <https://doi.org/10.3390/ijms17071164>
- Vaish, M., 2007. Mismatch repair deficiencies transforming stem cells into cancer stem cells and therapeutic implications. *Molecular Cancer* 6, 26. <https://doi.org/10.1186/1476-4598-6-26>
- Vaish, M., Mandhani, A., Mittal, R., Mittal, B., 2005. Microsatellite instability as prognostic marker in bladder tumors: a clinical significance. *BMC Urology* 5, 2. <https://doi.org/10.1186/1471-2490-5-2>
- Viswanathan, S., Shi, Y., Galipeau, J., Krampera, M., Leblanc, K., Martin, I., Nolte, J., Phinney, D.G., Sensebe, L., 2019. Mesenchymal stem versus stromal cells: International Society for Cell & Gene Therapy (ISCT®) Mesenchymal Stromal Cell committee position statement on nomenclature. *Cytotherapy* 21, 1019–1024. <https://doi.org/10.1016/j.jcyt.2019.08.002>
- Vitale, I., Manic, G., De Maria, R., Kroemer, G., Galluzzi, L., 2017a. DNA Damage in Stem Cells. *Molecular cell* 66, 306–319. <https://doi.org/10.1016/j.molcel.2017.04.006>
- Vitale, I., Manic, G., De Maria, R., Kroemer, G., Galluzzi, L., 2017b. DNA Damage in Stem Cells. *Molecular Cell* 66, 306–319. <https://doi.org/10.1016/j.molcel.2017.04.006>
- von Zglinicki, T., 2002. Oxidative stress shortens telomeres. *Trends in biochemical sciences* 27, 339–344.
- Weeden, C.E., Asselin-Labat, M.-L., 2018. Mechanisms of DNA damage repair in adult stem cells and implications for cancer formation. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* 1864, 89–101. <https://doi.org/10.1016/j.bbadis.2017.10.015>
- Wei, K., Kuchlerlapi, R., Edelmann, W., 2002. Mouse models for human DNA mismatch-repair gene defects. *Trends in molecular medicine* 8, 346–53. [https://doi.org/10.1016/S1471-4914\(02\)02359-6](https://doi.org/10.1016/S1471-4914(02)02359-6)
- Weyand, B., Eberle, I., n.d. Mesenchymal stem cells : basics and clinical application II.
- Wilson, A., Shehadeh, L.A., Yu, H., Webster, K.A., 2010. Age-related molecular genetic changes of murine bone marrow mesenchymal stem cells. *BMC Genomics* 11, 229. <https://doi.org/10.1186/1471-2164-11-229>
- Wong, R.S.Y., 2011. Mesenchymal stem cells: angels or demons? *BioMed Research International* 2011.
- Xi, J., Yan, X., Zhou, J., Yue, W., Pei, X., 2013. Mesenchymal stem cells in tissue repairing and regeneration: Progress and future. *Burns & trauma* 1, 13–20. <https://doi.org/10.4103/2321-3868.113330>
- Yang, S.-R., Park, J.-R., Kang, K.-S., 2015. Reactive Oxygen Species in Mesenchymal Stem Cell Aging: Implication to Lung Diseases. *Oxidative medicine and cellular longevity* 2015, 486263. <https://doi.org/10.1155/2015/486263>
- Yu, H., 2002. ENVIRONMENTAL CARCINOGENIC POLYCYCLIC AROMATIC HYDROCARBONS: PHOTOCHEMISTRY AND PHOTOTOXICITY. *Journal of Environmental Science and Health, Part C* 20, 149–183. <https://doi.org/10.1081/GNC-120016203>
- Yu, K.-R., Lee, J.Y., Kim, H.-S., Hong, I.-S., Choi, S.W., Seo, Y., Kang, I., Kim, J.-J., Lee, B.-C., Lee, S., Kurtz, A., Seo, K.-W., Kang, K.-S., 2014. A p38 MAPK-Mediated Alteration of COX-2/PGE2 Regulates Immunomodulatory Properties in Human Mesenchymal Stem Cell Aging. *PLoS ONE* 9, e102426. <https://doi.org/10.1371/journal.pone.0102426>
- Zhang, D., Wang, H., Tan, Y., 2011. Wnt/ β -Catenin Signaling Induces the Aging of Mesenchymal Stem Cells through the DNA Damage Response and the p53/p21 Pathway. *PLoS ONE* 6, e21397. <https://doi.org/10.1371/journal.pone.0021397>
- Zhang, J., Sun, Q., Zhang, Z., Ge, S., Han, Z.-G., Chen, W.-T., 2013. Loss of microRNA-143/145 disturbs cellular growth and apoptosis of human epithelial cancers by impairing the MDM2-p53 feedback loop. *Oncogene* 32, 61–69. <https://doi.org/10.1038/onc.2012.28>

Figure1. DNA repair



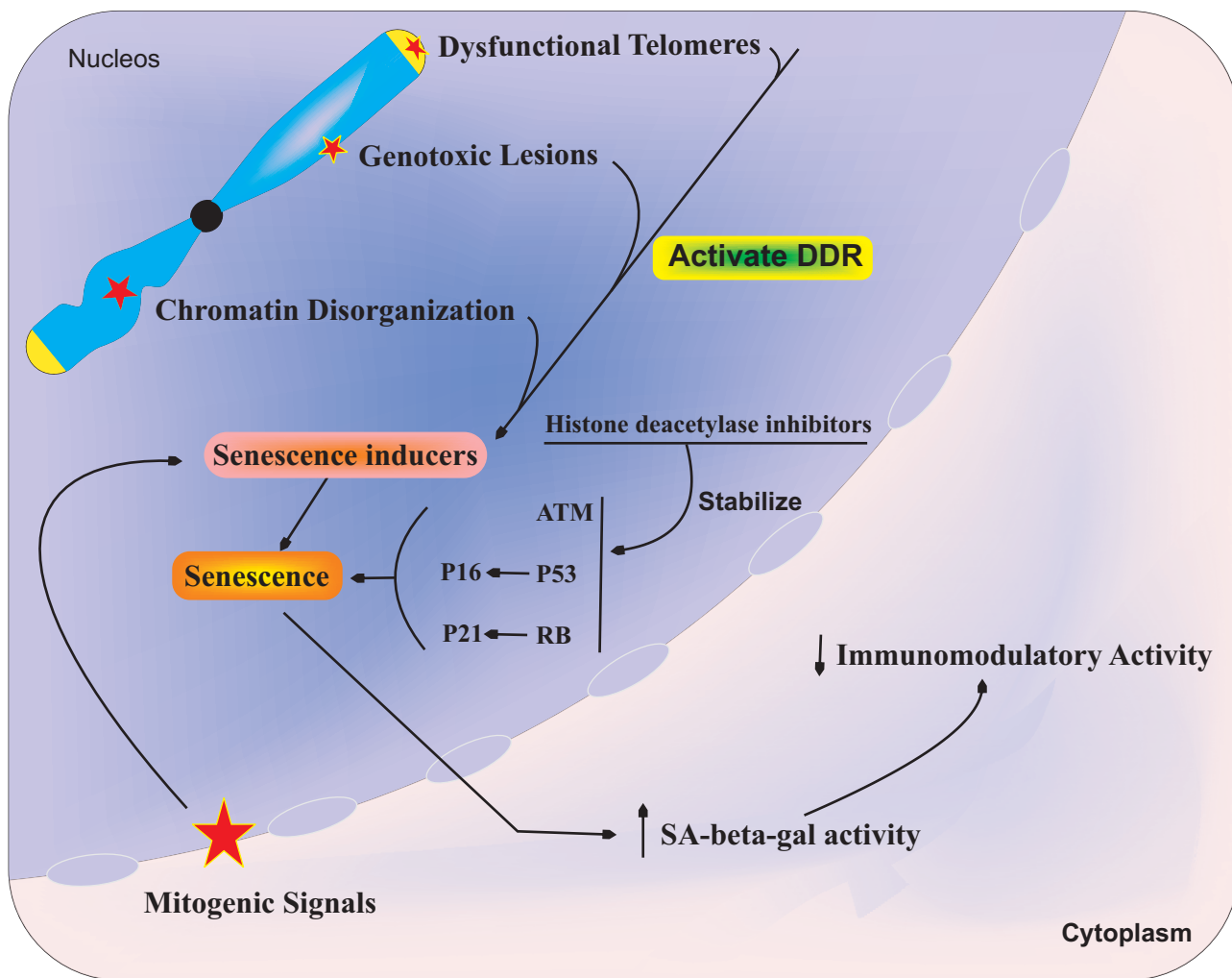


figure 2- senecence in MSC