Role of Stem Cells and Reactive Oxygen Species in Cancer

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An Insight into Nrf2 Signaling

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Abstract

Cancer cells produce excessive reactive oxygen species (ROS) than normal cells because of their increased metabolism and mitochondrial dysfunction. Accumulation of intracellular ROS generation activates proto-oncogenes and inactivates the funtions of tumor suppressor gene. Intracellular ROS act as important signaling molecules to activate cancer cells proliferation and their metastasis. Nuclear factor erythroid 2-related factor 2 (Nrf-2) plays a crucial role by regulating the antioxidant genes expression. Nrf-2 activation intracellularly protects both normal and cancer cells from oxidative damage. Generally, intracellular ROS

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S. Chakraborti (ed.), *Handbook of Oxidative Stress in Cancer: Therapeutic Aspects*,
https://doi.org/10.1007/978-981-16-5422-0_103

stimulate Nrf-2 signaling and increase the expression of cytoprotective antioxidant enzymes. In normal cells, enzymic antioxidants protect and nullify the intracellular oxidative imbalance. However, in tumor cells, oncogenic signaling activates Nrf-2 pathway, leading to tumor cells proliferation, drug resistance, stress adaptation, and activation of metabolic reprogramming. Nrf-2 activation has been reported in esophageal, liver, prostate, and breast cancer cells to defend the cancer cells against oxidant-induced cytotoxicity. Therefore, Nrf-2 inhibitors are used in anticancer agents. However, on the other hand, studies have shown that Nrf-2 transient expression is responsible to overcome chemical carcinogenesis. Nrf-2 signaling promotes proliferation, self-renewal, differentiation, and survival of cancer stem cells. On the other hand, stem cells transplantation has been tried for cancer therapy. Nrf-2 activation regulates self-renewal, quiescence, and regenerative potential of adult tissue stem cells and protects them from cellular stress and aging. Therefore, Nrf-2 activators are also reported to act as anticancer agents; however, Nrf2 activation is also a reason for cancer cells chemoresistance. Thus, the role of Nrf-2 signaling in cancer is remain inconclusive.

Keywords

Cancer stem cells · Reactive oxygen species · Mesenchymal stem cells

Introduction

Mesenchymal stromal/stem cells (MSCs) are spindle-shaped fibroblast-like cells that possess the capacity to self-renew with high proliferative ability (Ezhilarasan et al. 2021). MSCs exist in almost all tissues of the body and are commonly isolated from bone marrow. Clinically, bone marrow-derived MSCs hold challenging properties such as low yield, pain, and morbidity (Via et al. 2012), and this has led to isolate them from other sources like trabecular and cortical bone, amniotic fluid, synovial membranes, menses blood, umbilical cord blood, adipose tissue, endometrial polyps, skin, tendons, periosteum, skeletal muscle, peripheral blood, Wharton's jelly, etc. (Mushahary et al. 2018). MSCs are identified as progenitors in the outermost layers of larger arteries, veins, and the tunica adventitia (Corselli et al. 2012). MSCs exist as precursor cells in vascular/perivascular niches such as myogenic endothelial cells (intima), pericytes (media), and adventitial cells (adventitia) (Chen et al. 2015). MSCs are isolated (only 0.001–0.01%) from bone marrow by density gradient centrifugation. Approximately, one gram of 5×10^3 stem cells are isolated from one gram of adipose tissue, which is 500 times higher than the yield obtained from bone marrow. Peripheral blood-MSCs display a colony-forming efficiency ranging from 1.2 to 13 per million mononuclear cells (Hass et al. 2011). MSCs are multipotent progenitor cells with multi-lineage potential to differentiate into several cell types of mesodermal cell lineages, such as adipocytes, osteocytes, chondrocytes, myocytes, and cardiomyocytes (Ullah et al. 2015). MSCs are also differentiated into non-mesodermal origin, such as hepatocytes (endodermal) and neurons (ectodermal) (Al Ghrbawy et al. 2016). The Mesenchymal and Tissue Stem Cell Committee of the

International Society for Cellular Therapy has developed the minimum criteria of MSCs to universally define human MSCs: a) plastic-adherent and able to produce colony-forming unit fibroblast, b) must be phenotypically positive (>95%) for surface antigens such as cluster of differentiation (CD) 105, CD73, and CD90, and negative for (<5%) CD45, CD34, CD14 (or CD11b), CD79alpha (or CD19), and human leukocyte antigen–antigen D related (HLA-DR), c) capable of tri-lineage (adipocytes, chondroblasts, and osteoblasts) differentiation *in vitro* under specific culture conditions (Najar et al. 2017).

Genes related to developmental processes and stem cell functions are highly upregulated in adult MSCs. For instance, human primary MSCs were found to upregulate the expression of genes involved in embryogenesis such as LIM protein, distal-less homeo box 5, inhibitor of DNA binding 3, and eyes absent homolog 2 and genes responsible for neural development indicating the multi-lineage differentiation potentials of MSCs (Brendel et al. 2005). MSCs hold promising features that make them useful for cell therapies to treat several degenerative disorders. Firstly, MSCs have multi-lineage differentiation potential to develop into a variety of cells; secondly, MSCs have immunomodulatory potential by secreting variety of soluble factors; thirdly, systemically transplanted MSCs are shown to migrate towards sites of tissue injury and tumor microenvironments. Fourthly, MSCs contribute to regeneration of tissues and organs by their paracrine signaling (Christ et al. 2017). These are unique trophic properties MSCs have allowed to a potential advantage over targeted therapy. Their ease of accessibility, self-renewal abilities, versatile differentiation, multi-potency, and paracrine effect suggest use of MSCs may be ideal candidates for the treatment of various diseases (Ullah et al. 2015). Studies on these lines demonstrated MSCs against various diseases in experiential models and came out with promising results (Amer et al. 2018). In the clinical setting, cell therapy using MSCs have also been investigated against cardiovascular diseases, liver diseases, autoimmune diseases, hemophilia A, neurological disorders, etc. (Harris et al. 2018). MSCs, neural stem cells and hematopoietic stem cells (HSC) are often used in cancer treatment. Especially, HSC transplantation clically showed promissing results in the treatment of hematologic cancers like leukemia, multiple myeloma, and lymphomas. However, bone marrow stem cells isolation and stem cell therapy possess several lingering issues such as invasiveness, morbidity, and low yield after isolation, which has led to look for alternative sources for MSCs. However, bone marrow-derived cells have been widely used for therapeutic purpose in human. It has to underline that as on August 2021, there are more than 4000 clinical trials have been conducted or in process with MSCs against different types of cancer (http://www.clinicaltrials.gov), which further shows the promising therapeutic potentials of MSCs. Therefore, this chapter discusses the therapeutic potential of MSCs on ROS-induced cancers.

Stem Cell Therapy

Mesenchymal stem cells have been considered as a promising cell type for cell therapy due to their plasticity, migratory, and paracrine activity, regenerative and immunomodulation properties (Guo et al. 2018). The safety and therapeutic

potential of MSCs-based therapies have been investigated clinically against diabetes, different types of cancer, respiratory, liver, heart, neurodegenerative, bone and graft-vs-host diseases, Crohn's disease, autoimmune diseases, and several others (Campbell et al. 2015). MSCs have the ability to secrete a wide range of growth factors and cytokines such as interleukins (ILs), platelet-derived growth factor, hepatocyte growth factor, vascular endothelial growth factor, fibroblast growth factor, insulin growth factor, matrix metalloproteinases, etc. which can influence cells responsible for cell recruitment and differentiation, angiogenesis and tissue regeneration, immunomodulation properties (Farzaneh et al. 2018). Ample evidence suggests that therapeutic potentials of MSCs are attributed to their multi-lineage differentiation, paracrine and tropism properties (Farzaneh et al. 2018). MSCs have promising immunoregulatory potential, and they potentially inhibit immune cells differentiation, maturation, function, and their proliferative response which makes them suitable for the solid organ transplantation (Kaundal et al. 2018). MSCs indirectly regulate the T and B cells function via dendritic cells modulation and thereby prevent the acute graft versus host disease (Česen Mazič et al. 2018). MSCs also interfere with other innate immune cells such as macrophages, natural killers, neutrophils, eosinophils, and mast cells. During allogeneic islet transplantation, MSCs suppress dendritic cells function (Aldinucci et al. 2010). These properties make MSCs attractive option for solid organ transplantation (Holt 2017). Currently, a randomized phase 1 trial is ongoing to study the effect of MSCs on immune modulation in kidney transplanted patients (NCT02409940). Both resident and circulating macrophages recruitment cause inflammation in the disease microenvironment. After cell transplantation, MSCs educate macrophages to alter their phenotype from M1 pro-inflammatory to M2 anti-inflammatory phenotype (Bouchlaka et al. 2017). Thus, MSCs regulate tissue repair and regeneration via their immunomodulatory role.

Stem Cells in Cancer Therapy

Hematopoietic stem cells (HSCs), MSCs, and neural stem cells (NSCs) are frequently used in cancer treatment. Bone marrow-derived HSCs can form mature blood cells, and hence, FDA has approved HSCs for the treatment of blood cancers such as multiple myeloma, leukemia, and other blood-related disorders (Copelan 2006). As aforementioned, MSCs have exceptional biological properties and have been widely used to deliver drugs, nanoparticles, and nucleic acids for treating a variety of cancers (Lin et al. 2019). NSCs are existing in central nervous system and they have self-renewing properties and they can generate nerve cells such as glial cells and neurons. NSCs have been widely tested against primary and metastatic cancer models (Kanojia et al. 2015). Stem cells have characteristic homing properties. They can swiftly migrate to defined stem cell niches in bone marrow, where HSCs undergo the engraftment process prior to giving rise to specialized blood cells. The active interaction between C-X-C motif chemokine receptor (CXCR) 4 receptor of stem cell with gradient SDF (stromal cell-derived factor)-1 secreted from cells in

bone marrow niches was revealed as the molecular mechanism of HSCs homing towards bone marrow. Further, ceramide-1 phosphate, sphingosine-1-phosphate mediated signaling and extracellular adenosine triphosphate or uridine 5-′-triphosphate and calcium and hydrogen ions are also reportedly involving homing properties of HSCs (Chu et al. 2020). Cancer cells educate MSCs to become cancer associated MSCs, and these cancer-associated MSCs attain pro-metastatic behavior in the tumor microenvironment (TME). Cancer (breast, prostate, multiple myeloma, and osteosarcoma) cells express SDF-1, chemokine (C-X-C motif) ligand 16, chemokine (C-C motif) ligand 25, and interlukin-6 in TME. These cytokines and chemokines are implicated in stem cells tropism properties by attracting stem cells to the TME (Lourenco et al. 2015). Therefore, due to their cancer-specific tropism, stem cells are used to carry anticancer drugs (Takayama et al. 2021). Once stem cell reaches TME, they interact with cancer cells through direct and indirect molecular mechanisms that affect tumor development. For instance, MSCs secrete a variety of soluble and paracrine factors, and extracellular vesicles contain several anticancer contents such as mRNA, miRNA, long non-coding RNAs, etc. (Chang et al. 2021). Accumulating evidences have demonstrated that stem cells-mediated paracrine mechanism may affect tumor survival, progression, and metastasis. For instance, MSCs inhibits cancer cell proliferation signaling pathways such as phosphatidylinositol 3-kinase (PI3K)/protein kinase B and induced cell cycle arrest in tumor cells (Lu et al. 2019). In contrast, MSCs also contribute to tumor progression by differentiating into cancer-associated fibroblast in TME (Papait et al. 2020). Overwhelming evidences suggest that crosstalk between MSCs and tumor cells in TME results in tumor-promoting and tumor-suppressing effects, and thus MSCs are also considered as double-edged sword (Hmadcha et al. 2020). Therefore, clinical use of MSCs in oncology is still subjected to debate.

The cancer-promoting and tumor-suppressing/therapeutic potential of MSCs was reported to depend on the source of MSCs, dose and duration of MSCs treatments, death of stem cells after transplantation, tumor models, cell delivery method, and other experimental conditions, and these discrepancies must be addressed in the near future. A few studies have reported the role of MSCs in ROS-induced cancer. For instance, elderly MSCs accumulate ROS, which hinder their differentiation functions (Khanh et al. 2020). MSCs-derived stanniocalcin-1 (STC1) prolongs the survival of lung cancer cells (A549) by reducing intracellular ROS, uncoupling oxidative phosphorylation, and enhancing the Warburg effect. This study shows that MSCs can protect cancer cells from ROS-induced apoptosis via STC1 secretion (Ohkouchi et al. 2012). ROS induces proinflammatory mediators in TME. MSCs can be recruited from BM and they have tropism for TME. In TME, MSCs induce peripheral tolerance, promoting cancer cell survival (Jung et al. 2013). ROS is responsible for MSCs recruitment via enhancing inflammatory signaling activation in prostate cancer. In a study, oxidative stress suppression by antioxidant (*N*-acetyl cysteine) was shown to inhibit MSC recruitment by reducing NF-κB signaling in prostate cancer (Yu et al. 2018). However, in an *in vivo* study, intrarectal transplantation of BM-MSCs improved oxidative stress-mediated colorectal cancer induced by 1, 2-dimethylhydrazine (DMH) in rats. Reduced apoptotic markers such as caspase 3 and cytochrome c level was reported

after BM-MSCs transplantation in DMH-intoxicated rats suggesting the antiapoptotic role of MSCs (Alkhuriji et al. 2021).

ROS in Cancer

The reactive oxygen species (ROS) are implicated in myriad human ailments, including cancer. ROS are naturally generated as a by-product of several biological activities, especially oxygen metabolism (Ezhilarasan 2018). The oxygen-containing species classified as ROS, which includes alkoxyl, superoxide, hydroxyl, hydroperoxyl, peroxyl radicals, and non-radicals such as hydrogen peroxide, hypochlorous acid, and ozone (Halliwell 1996). ROS also function as intracellular secondary messengers in cell signaling and are required for a variety of biological activities in both normal and cancerous cells (Chio and Tuveson 2017). A closely controlled equilibrium between the generation of ROS by oxidizing systems and the removal of ROS by antioxidant systems maintains ROS homeostasis in cells. Oxidative stress occurs when this equilibrium is disrupted by overwhelming ROS accumulation or reduced intracellular antioxidant activity (Veal et al. 2007). Numerous exogenous (chemically induced stress) and endogenous (mitochondrial stress) factors are responsible for the intracellular ROS accumulation. Mitochondria, one of the major sources of high-level endogenous ROS generation, causes the oxidative stress in cells (Starkov 2008). ROS is produced largely in the electron transport chain in the inner mitochondrial membrane by oxidative phosphorylation, a process that creates adenosine triphosphate (ATP) from oxygen and simple carbohydrates (Li et al. 2013). Several enzymes such as cyclooxygenase, lipoxygenase, NADPH oxidase, xanthine oxidase, and nitric oxide synthase are responsible for intracellular ROS production. Biochemical metabolic processes such as microsomal enzymes mediated biotransformation reactions, one carbon metabolism, β-oxidation and glutaminolysis are often involve in the intracellular ROS liberation (Jiang et al. 2011). Mitochondria produce superoxide and H_2O_2. To defend themselves from the damaging effects of ROS, cells have evolved antioxidant mechanisms that allow them to detoxify themselves. SOD, catalase (CAT), glutathione peroxidases (GPx), peroxiredoxins (PRDX), and glutaredoxins (GLRX) are intracellular antioxidant enzymes and vitamins A, C, D, E, β-carotene, and ubiquinone are non-enzymatic antioxidant systems (Zuo et al. 2015).

Cancer cells produce more ROS than normal cells because of their augmented metabolism and mitochondrial malfunction (Ezhilarasan et al. 2020a). The high level of ROS generation in cancer cells is a biochemical and molecular alteration that is required for carcinogenesis, metastasis, and drug resistance (Elumalai et al. 2021). ROS are thought to play oncogenic roles by contributing to activation of proto-oncogenes and inactivation of tumor suppressor genes and by also acting as important signaling molecules to induce abnormal cell growth and metastasis. According to growing evidence, ROS in cancer cells are considered as "double-edged sword." As signaling mediators, ROS promotes cancer cell proliferation, metastasis, and drug resistance at lower levels (Sohaib and Ezhilarasan 2020). At the same time, sufficient amounts of ROS are necessary for cancer cell homeostasis, which is

implicated in cellular activities including proliferation, differentiation, migration, and induced apoptosis. On the other hand, high concentrations of ROS are toxic to cancer cells and induce cell death by causing damage to proteins, nucleic acids, lipids, membranes, and organelles (Raj et al. 2020). Therefore, in vitro anticancer studies demonstrated that cancer cells (liver, lung, oral, and colon) proliferation and function can be decreased through intracellular ROS induction by chemical and natural anticancer agents (Abijeth and Ezhilarasan 2020; Raj et al. 2020; Nandhini et al. 2020; Rithanya and Ezhilarasan 2021; Shathviha et al. 2021).

ROS can directly damage the DNA and generate a DNA double-strand break, which causes the production of mutagenic 8-oxo-7-hydro-2′-deoxyguanosine (8-oxodG). Because of its ability to couple with both cytosine and adenine, 8-oxodG promotes guanine to thymine transversion; it is a major cause of random mutagenesis (Oka and Nakabeppu 2011). As a result, the aggregation of 8-oxodG in cellular genomes causes cancer. Iron is a significant generator of ROS, and it is becoming recognized as a key factor to induce cell death in a range of organisms and clinical conditions, notably, iron-induced oxidative stress was also implicated in the pathogenesis of a variety of malignancies (Anirudh and Ezhilarasan 2021). Approaches to inhibit or stimulate ROS in cancer cells tend to be potential therapies due to the bidirectional feature of ROS. Antioxidants are often thought to be useful for cancer treatment and prevention because they can reduce oxidative stress by quenching ROS levels (Ezhilarasan 2018). In fact, suppressing intracellular ROS with deferasirox (DFX), an oral iron chelator, caused death in multiple myeloma (MM) cells. DFX works against MM by inhibiting proline-rich tyrosine kinase 2 phosphorylation and reducing the generation of ROS (Kamihara et al. 2016). Recent studies have been approached on ROS-dependent promotion of cellular proliferation. The finding of ROS as second messengers involved in growth factor-mediated activation of PI3K/AKT/mechanistic target of rapamycin and mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) mitogenic signaling cascades is one of the most important achievements. The reversible inhibition of phosphatase and tensin homolog (PTEN) caused by thiol-disulfide transformation in response to H_2O_2 therapy led researchers to look into the mechanism of ROS in neoplastic development (Lee et al. 2002). Abundant ROS produced by NOX following insulin stimulation induces oxidative deactivation of PTEN and activation of PI3K/AKT in neuroblastoma cells. Insulin-induced phosphorylation of PI3K/AKT is significantly decreased in neuroblastoma cells pretreated with the NOX inhibitor diphenyleneiodonium before insulin stimulation (Calvo-Ochoa et al. 2017). Furthermore, ROS activates MAPKs by suppressing the MAPK and c-Jun N-terminal kinases (JNK) enzymes via reversible oxidation of sulfenic acid in catalytic site of cysteine that causes activation of JNK (Kamata et al. 2005).

ROS and Nuclear Factor Erythroid 2-Related Factor 2 (Nrf-2) Signaling

Nrf-2 belongs to the family of Cap ‘n’ Collar transcription factor, comprises of 605 amino acids and it is classified into Neh1-Neh7 highly conserved functional

domains (Süntar et al. 2021). Intracellularly, Nrf-2 plays a vital role in redox hemostasis by regulating the expression of cytoprotective antioxidant genes that eventually exerts anti-inflammatory functions. Nrf-2 characteristically exists in the cytosolic domain through association with a cytosolic actin-binding protein and the E3 ligase adaptor, Kelch-like ECH-associated protein 1 (Keap1), a principal negative regulator of Nrf-2 (Krajka-Kuźniak et al. 2017). Keap 1 is a cysteine-rich protein (contains 27 cysteine residues in humans), comprises of 624 amino acids (Kansanen et al. 2009) and plays a central role in the Nrf-2 regulation and activity. Intracellularly, Keap1 occurs as dimers and functions as a substrate linker protein for the interaction of Cul3/Rbx1-based E3-ubiquitin ligase complex with Nrf-2, leading to the continuous ubiquitination of Nrf-2 and its subsequent proteasomal degradation (Li et al. 2019). Thus, the continuous Nrf-2 degradation under basal conditions keeps the low level of Nrf-2 to regulate antioxidants. When cells encounter mild intracellular oxidative stress by overwhelming ROS accumulation, or due to chemically induced stress, Nrf-2 stabilizes after dissociation from Keap 1 and translocates to the nuclei. Inside the nuclei, Nrf-2 interacts with small musculoaponeurotic fibrosarcoma (sMaf), and binds to ARE, leading to the increased transcription of antioxidant genes responsible for the antioxidant enzymes such as NAD(P)H quinone oxidoreductase 1 (NQO1), heme oxygenase-1 (HO-1), and superoxide dismutase 2 (SOD2), catalase, and glutathione peroxidase (Ezhilarasan et al. 2016). Nrf-2 has cytoprotective activity and it has positive and negative effects on cancer. As aforementioned, in healthy cells, under normal conditions, Keap1-mediated proteasomal degradation inhibits Nrf-2 level, and in stress condition Nrf-2 dissociates from Keap1, translocates to nucleus, and activates cytoprotective gene expression responsible for intracellular antioxidant response. Thus, the cellular antioxidant system is mainly controlled by Nrf-2 and its related genes, which are stimulated and differentially expressed in cancer to enhance cancer cell survival (Sporn and Liby 2012).

An excessive ROS level alters the gene expression through variety of signaling pathways. Cancer cells react to oxidative stress by increasing the transcription of antioxidant enzymes to avoid additional intracellular ROS (Perillo et al. 2020). Nrf-2 activation has been reported in different cancer types to defend the cancer cells against oxidant-induced cytotoxicity (DeNicola et al. 2011). Nrf-2 has been found to stimulate the growth of esophageal cancer cells to detoxify ROS (Kitano et al. 2018). Enhanced Nrf-2 is a poor prognostic sign in human hepatocellular cancer (Zhang et al. 2015). Upregulation of Nrf-2 sensitizes prostate cancer cells to radiation by lowering baseline ROS levels (Liu et al. 2015). The downregulation of hypoxia-inducible factor 1 (HIF-1) by Nrf-2 activation in breast cancer cells may decrease the tumor's invasiveness and metastasis. In addition, HIF-1 expression was already found to be positively associated with the amount of ROS, and its increased expression has been implicated in tumor development and metastasis (Sarkar et al. 2016). In cancer cells, oncogenic signaling pathway causes constitutive Nrf-2 activation leads to cancer cell proliferation, drug resistance, stress adaptation, and activation of metabolic reprogramming and induces oncogenic gene expressions (Telkoparan-Akillilar et al. 2021). Nrf-2 activation intracellularly protects both

normal and cancer cells from oxidative damage (Raj et al. 2020). Nrf-2 activation also inhibits the malignant formation of normal cell. For instance, increased intracellular ROS stimulates Nrf-2 signaling, increasing the cytoprotective endogenous antioxidant enzymes such as NQO1, HO-1, and SOD 2. These enzymic antioxidants nullify the intracellular oxidative stress in normal cells. However, once cancer is initiated, intracellular antioxidants cause cancer therapies resistance by interrupting ROS-mediated cancer cell death (Mirzaei et al. 2021).

Nrf-2 Signaling in MSCs

As a cell stress sensor, Nrf-2 activation triggers the stem cells proliferation, differentiation, survival, and self-renewal (Dai et al. 2020). Nrf-2 overexpression significantly increased the proliferation and reduced the apoptotic rate in human umbilical cord-derived MSCs. Interestingly, Nrf-2 knockdown impaired the stem cell markers expression and osteogenic differentiation under cobalt chloride-induced hypoxic conditions (Yuan et al. 2017). Nrf-2 regulates pluripotency factors, metabolism, redox homeostasis, and cellular stress responses in pluripotent stem cells. Nrf-2 regulates self-renewal, quiescence, and regenerative capacity of adult tissue stem cells and protects them from cellular stress and aging (Dai et al. 2020). In a study, Nrf-2 bearing recombinant virus was prepared in appropriate mammalian cell line to infect BM-MSCs. Nrf-2 producing BM-MSCs showed improved anti-oxidative and anti-apoptotic capabilities than unmodified BM-MSCs *in vitro*. Transient expression of Nrf-2 by BM-MSCs protected them by expressing SOD and HO-1 enzymes against cell death and the apoptosis against experimentally induced hypoxic and oxidative stress conditions. This strategy can be used to avoid graft cell death due to hypoxia, oxidative stress, and serum deprivation during MSC-based cell therapy (Mohammadzadeh et al. 2012). In human placental MSCs of fetal origin (hfPMSCs), inhibition of Nrf-2 signaling led to decrease in caspase-3 expression in human lung cancer cells (A549). Further, hfPMSCs protected the H_2O_2-induced cell oxidative injury by regulating the Nrf-2-Keap1-ARE signaling-mediated cell apoptosis (Yan et al. 2019). This strategy can also be useful to reduce oxidative environment in cancer cells to improve cancer therapy (Fig. 1).

Nrf-2 Signaling in Cancer Stem Cells

Cancer stem cells (CSCs) are subpopulations of cells found within the tumor mass, and they have definite role in tumorigenesis, metastasis, anticancer drug resistance, and tumor relapse after therapy (Ezhilarasan et al. 2020a). Nrf-2 signaling promotes proliferation, differentiation, survival, and self-renewal of CSCs (Kahroba et al. 2019). Redox homeostasis is a vital governing factor for cancer stemness, which is mainly under the control of Nrf2 signaling. In general, CSCs have low endogenous

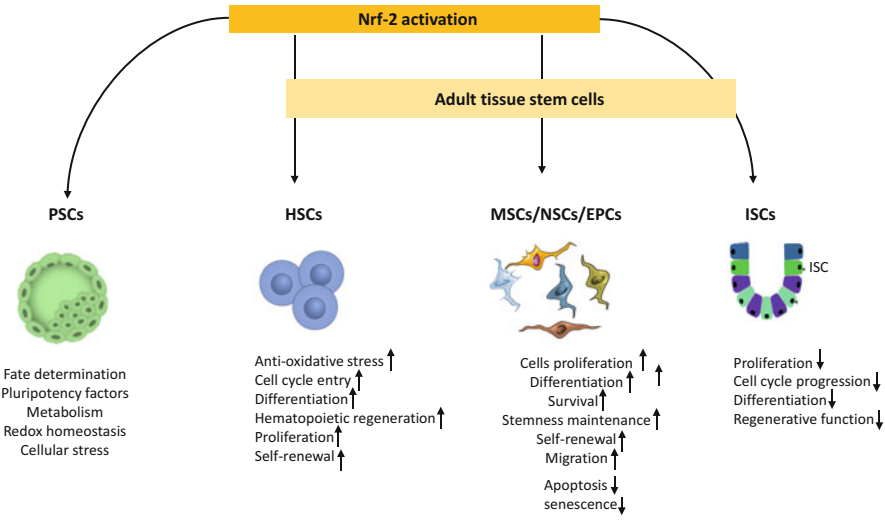

Fig. 1 Effect of Nrf-2 activation in pluripotent stem cells (PSCs) and adult tissue stem cells. Up arrows indicate the increased response and down arrows indicate the decreased response. EPC, endothelial progenitor cells; HSCs, hematopoietic stem cells; MSCs, mesenchymal stem cells; NSCs, neural stem cells; ISCs, intestinal stem cells

ROS levels as compared to non-CSCs. Such a low-endogenous ROS helps in promoting CSCs tumorigenicity and stemness (Chang et al. 2014). Long-term anticancer drugs treatments converts breast cancer cells to CSC-like cells with upregulation of antioxidant enzymes and low ROS levels. Nrf-2 activation was observed in these breast cancer cells with CSC-like properties, suggesting the possible role of Nrf-2 in the establishment of CSCs (Achuthan et al. 2011). For instance, activation of Nrf-2 signaling in CSCs provokes the glycolytic gene expression and inhibits TCA cycle by activating pyruvate dehydrogenase kinase 1 to activate Warburg effect to minimize ROS production in mitochondria via GRP78/p-PERK/Nrf-2 signaling pathway, leading to the CSCs stemness maintenance by reduced mitochondrial ROS (Chang et al. 2018). Colon CSCs-like cells were shown to have Nrf-2 activation-induced antioxidant and detoxifying proteins in total secretome, which reveals the crucial role of Nrf-2 in maintenance of CSCs metabolic status (Emmink et al. 2013). Nrf-2 activation leads to the upregulation of anti-apoptotic markers in CSCs. For instance, activation of Nrf-2 signaling in cervical CSCs is reported to induce the ATP-binding cassette transporter G2 drug transporter, Bcl-2 (anti-apoptotic protein), and B-cell-specific Moloney murine leukemia virus integration site-1 (a stem cell marker) expressions, leading to tumorigenesis and resistance to therapies (Jia et al. 2015). Therefore, Nrf-2 inhibitors are tried in anticancer therapies (Panieri and Saso 2019). The Nrf-2 activation-related events in cancer cell is presented in Fig. 2.

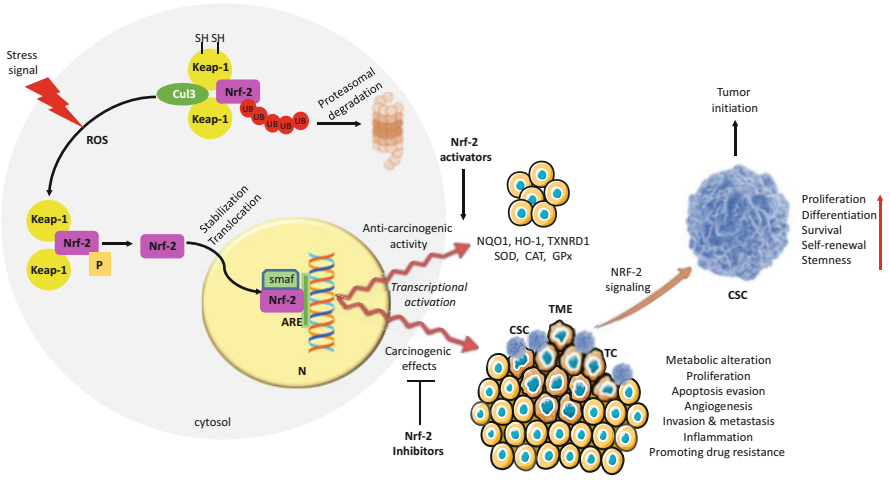

Fig. 2 NRF-2 signaling in cancer cells. ROS, reactive oxygen species; NAD(P)H quinone oxidoreductase 1 (NQO1), TXNRD1, thioredoxin reductase 1; heme oxygenase-1 (HO-1) and superoxide dismutase 2 (SOD2), CAT, catalase; GPx, glutathione peroxidase; Keap-1, Kelch-like ECH-associated protein 1; Cul3, cullin-3; Smaf, small musculoaponeurotic fibrosarcoma; ARE, antioxidant response element; TME, tissue microenvironment; CSC, cancer stem cells; TC, tumor cells; N, nucleus

Conclusion

Nrf-2 regulates the expression of many cytoprotective antioxidant genes and proteins involved in cell proliferation and several other physiological process. Activation of Nrf-2 pathway was shown to involve in tumor initiation, dissemination, and cancer therapies resistance. Nrf-2 activation is important to maintain stemness properties in CSCs and Nrf-2 signaling-activated CSCs involve in tumor cell invasion, metastasis, and chemoresistance. *In vitro* tumor cell lines exhibit upregulation of Nrf-2 expression, and similarly, increased Nrf-2 expression is also reported in human breast, esophagus, endometrial, lung, and prostate tumors biopsies. Therefore, Nrf-2 inhibitors are used in anticancer therapies. On the other hand, Nrf-2 activation increases the proliferation, differentiation, survival, stemness maintenance, self-renewal, and migration in adult tissue stem cells. Nrf-2 activation also regulates stemness and fate of pluripotent stem cells. Transient Nrf-2 expression was helpful to overcome chemical carcinogenesis. Therefore, Nrf-2 activators are also considered potential antitumor agents; however, Nrf-2 overexpression also reportedly induces chemoresistance in tumor cells. Thus, Nrf-2 plays a differential role in normal, cancer, and stem cells. Therefore, Nrf-2 signaling pathways must be targeted based on the specific cell types and pathology.

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