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Review

# The role of DNA repair in the pluripotency and differentiation of human stem cells

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ABSTRACT

All living cells utilize intricate DNA repair mechanisms to address numerous types of DNA lesions and to preserve genomic integrity, and pluripotent stem cells have specific needs due to their remarkable ability of self-renewal and differentiation into different functional cell types. Not surprisingly, human stem cells possess a highly efficient DNA repair network that becomes less efficient upon differentiation. Moreover, these cells also have an anaerobic metabolism, which reduces the mitochondria number and the likelihood of oxidative stress, which is highly related to genomic instability. If DNA lesions are not repaired, human stem cells easily undergo senescence, cell death or differentiation, as part of their DNA damage response, avoiding the propagation of stem cells carrying mutations and genomic alterations. Interestingly, cancer stem cells and typical stem cells share not only the differentiation potential but also their capacity to respond to DNA damage, with important implications for cancer therapy using genotoxic agents. On the other hand, the preservation of the adult stem cell pool, and the ability of cells to deal with DNA damage, is essential for normal development, reducing processes of neurodegeneration and premature aging, as one can observe on clinical phenotypes of many human genetic diseases with defects in DNA repair processes. Finally, several recent findings suggest that DNA repair also plays a fundamental role in maintaining the pluripotency and differentiation potential of embryonic stem cells, as well as that of induced pluripotent stem (iPS) cells. DNA repair processes also seem to be necessary for the reprogramming of human cells when iPS cells are produced. Thus, the understanding of how cultured pluripotent stem cells ensure the genetic stability are highly relevant for their safe therapeutic application, at the same time that cellular therapy is a hope for DNA repair deficient patients.


**Contents**



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## 1. Introduction

Genetic material is constantly exposed to a variety of genotoxic agents that can produce lesions on DNA and ultimately generate genomic instability. The potential sources of DNA damage include (i) endogenous factors such as those generated by metabolic activities (e.g., reactive oxygen species [ROS]) and DNA replication and (ii) exogenous factors such as environmental agents (e.g., ultraviolet [UV] and ionizing radiation [IR]). Some DNA lesions create structural alterations in the DNA that can impair gene transcription and DNA replication, thereby compromising vital cellular functions [1].

To counteract the constant occurrence of DNA lesions, cells have evolved complex DNA repair systems that are responsible for maintaining the integrity of genetic material. The major excision repair mechanisms in most cells for repairing damaged or inappropriate base incorporation within single DNA strands are base excision repair (BER), nucleotide excision repair (NER) and mismatch repair (MMR) [2]. In addition, two important mechanisms are responsible for repairing lesions involving two strands of DNA, interstrand crosslinks (ICLs) or double strand breaks (DBS): non-homologous end-joining (NHEJ) and homologous recombination (HR) [3]. The repair of different types of DNA lesions therefore depends on different sets of proteins that presumably undergo crosstalk to form a network for protection of the cell genome [4]. DNA repair mechanisms are ubiquitous protective mechanisms comprised of several pathways that address many different types of DNA lesions.

Human stem cells have the potential to differentiate into several cell types. Adult stem (AS) cells are important in the long-term maintenance of tissues throughout life, as they are responsible for regenerating tissues in response to damage and for replacing senescent terminally differentiated cells. AS cells normally have their differentiation limited to certain derived tissues and do not generate germ cell lines. In contrast, human embryonic stem (ES) cells have the potential to differentiate into all cell types found in mammalian embryos, including germ cells. Although stem cells are difficult to obtain and their clinical use is limited by ethical and safety considerations, genetic strategies were developed to reprogram the nuclei of somatic differentiated cells into pluripotent stem cells, which were termed induced pluripotent stem (iPS) cells [5]. The maintenance of genomic integrity in stem cells, both by increased stress defense and DNA repair mechanisms is extremely robust because genetic alterations can potentially compromise the functionality of entire cell lineages. For AS cells, genetic alterations have been linked to the impairment of proliferation and differentiation capacity and to the increased potential of tumorigenicity and aging [6]. Unrepaired DNA damage can lead to genomic instability and mutation, which can affect cell proliferation control, resulting in cancer. The inability of cells to cope with DNA damage triggers certain cellular responses that may lead to cell death. This event is critical because it provokes a decrease in the pool of stem cells, reducing the body's ability to repopulate damaged tissues and leading to aging.

In this review, we discuss (i) the DNA repair capacity of stem cells and its crucial role in differentiation and pluripotency maintenance of ES and iPS cells; (ii) the DNA repair responses of cancer stem cells and their implications for cancer therapy; and (iii) the possible roles of DNA repair in the reprogramming process of somatic cells to a pluripotent state and the potential use of iPS cells for cellular therapy of DNA repair deficient patients.

## 2. Adult stem cells have elevated DNA repair capacity

AS cells have the remarkable ability of self-renewal and differentiation into different functional cell types. In contrast to postmitotic or short-lived somatic cells, tissue-specific stem cells persist and function throughout the entire life of an organism to regulate tissue homeostasis and regeneration. AS cells are responsible for supporting many tissue functions, including the production of undifferentiated cells for tissue rejuvenation [6]. The functional demands and the longevity of stem cells indicate that they are uniquely equipped to maintain genomic integrity in ways different from those of somatic cells. In fact, as it will be discussed here, AS cells generally display high levels of DNA repair capacity, which decreases with differentiation.

One of the most versatile and highly conserved DNA repair mechanisms is the NER. This pathway is involved in the repair of helix-distorting lesions such as UV-induced photoproducts, cyclobutane pyrimidine dimers (CPDs) and pyrimidine 6–4 pyrimidone photoproducts ((6–4)PPs) [7]. Many bulky DNA adducts, including ICLs, generated by environmental pollutants (e.g., aldehydes) or by anti-tumor agents (e.g., cisplatin) are also repaired by the NER pathway [8]. The NER pathway comprises two subpathways: global genome repair (GGR), which senses the distortion in the double helix and repairs damage throughout the genome, and transcription-coupled repair (TCR), which addresses DNA lesions at the transcribing strand of active genes.

NER activity was shown to decrease during the differentiation of many types of human stem cells [9]. When the repair of UV-induced DNA lesions was compared in terminally differentiated human (hNT) neurons and their precursor cells ($NT_2$), the removal of CPDs, by GGR, was markedly decreased in hNT neurons. Furthermore, (6–4)PPs repair kinetics was also perturbed in hNT neurons, since nearly complete removal was achieved only after 3 days, whereas a complete repair of (6–4)PPs lesions was observed within hours in NT2 cells [10]. The attenuation of GGR upon differentiation was also observed in differentiated macrophages as a result of the hypo-phosphorylation of the ubiquitinating enzyme E1. The TFIIH complex, a component of NER pathway, could be the potential target for ubiquitination and may represent an important mechanism to regulate NER upon differentiation [11]. By contrast, TCR subpathway remained functional in those cells and there was no significant reduction in levels of NER enzymes. Moreover, terminally differentiated cells also efficiently repair the nontranscribed strand of active genes, a mechanism known as differentiation-associated repair (DAR). In this case, NER enzymes are recruited at the transcription domain resulting in the efficient repair of the damaged non-transcribed DNA strands [12,13].

BER is responsible for correcting small base modifications (e.g., oxidized bases and alkylation damage) and DNA single-strand breaks (SSBs). Such damage may result from endogenous events (e.g., mitochondrial metabolism) or exogenous genotoxic agents (e.g., anticancer drugs). The BER pathway also comprises two subpathways: the short-patch subpathway in which a single nucleotide is replaced and the long-patch subpathway in which 2–13 nucleotides are replaced [14]. In situ hybridization studies showed that, in the mice brain, some BER glycosylases (such as OGG1 and Neil 1) are highly expressed in neural stem or progenitor cells, conferring high capacity to remove 8-oxoguanine (8-oxoGua) lesions, which were reduced upon the induction of differentiation [15]. The murine Nei endonuclease VIII-like 3 (Neil3) glycosylase was also found to be specifically expressed in areas known to harbor neural stem and progenitor cells [16,17]. In addition, other groups observed downregulation of BER genes, such as XRCC1 and DNA ligase III and DNA ligase I during differentiation of mouse myoblasts to terminally differentiated myotubes. Importantly, both short and long patch BER pathways were dysfunctional in myotubes and accumulation of 8-oxoGua was observed as a consequence. The attenuation of BER in these differentiated cells also resulted in the accumulation of SSBs and phosphorylated H2AX nuclear foci after exposure to hydrogen peroxide [18].