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Differentiation of Embryonic Stem Cells and Oxidative DNA Damage / DNA Repair Systems

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Abstract

To maintain genomic integrity, cells are equipped with a defense capacity against DNA damage generated in genomic DNA. The avoidance of mutations in genomic DNA is especially critical for undifferentiated cells, such as Embryonic stem (ES) cells. Among the many factors damaging DNA, Reactive Oxidative Species (ROS) are crucial and unavoidable, and thus the roles of ROS in cell differentiation and cellular functions have been extensively studied. However, the biological significance of ROS generation in cell differentiation remains a matter of debate. Among the types of DNA damage due to ROS, 8-oxoguanine (8-oxo-Gua) has been well studied and is known to generate GC-to-TA point mutations in genomic DNA. We recently analyzed the differences in the resistance to 8-oxo-Gua generation between undifferentiated cells and their differentiated counterparts. Our studies indicated that undifferentiated cells were more resistant to 8-oxo-Gua generation, in comparison to differentiated cells. In this short review, we describe the relationship between ES cell differentiation and oxidative DNA damage / DNA repair systems, by summarizing our previous work and that of other researchers.

Keywords: Cell differentiation; Embryonic stem cells; 8-Oxoguanine; 8-Oxoguanine DNA glycosylase 1; Reactive oxygen species

Abbreviations: BER: Base Excision Repair; ES cells: Embryonic Stem Cells; HPLC-ECD: High Performance Liquid Chromatography coupled with Electrochemical Detection; LIF: Leukemia Inhibitory Factors; MAPK: Mitogen Activated Protein Kinase; MUTYH: Homologue of Mut Y; MTH1: Homologue of Mut T; Nox: NADPH oxidase; NER: Nucleotide Excision Repair; NPR-C: Natriuretic Peptide Receptor-C; OGG1, 8-Oxoguanine DNA Glycosylase 1; 8-oxo-Gua: 8-Oxoguanine; PDGF-BB: Platelet-derived Growth Factor BB; ROS: Reactive Oxygen Species

Introduction

Embryonic stem (ES) cells are isolated during the blastocyst stage of embryonic development, and are pluripotent, self-renewing cells. Since ES cells are being considered for use in organ replacement and regeneration therapies, they must have an ability to prevent the generation and propagation of mutations in genomic DNA, generated as a consequence of DNA damage. Thus, before ES cells can be used in medical applications including therapy, the exact mechanisms by which their genomic integrity is maintained should be revealed.

Reactive oxygen species (ROS) are believed to play a crucial role in carcinogenesis, by generating oxidative DNA damage. Some forms of oxidative DNA damage have been recognized as pre-mutagenic base modifications, because they induce mutations in genomic DNA. 8-Oxoguanine (8-oxo-Gua) is a well studied form of DNA damage generated by ROS (Figure 1A), and is considered to play an important role in carcinogenesis [1,2]. Since 8-oxo-Gua causes GC-to-TA transversion type point mutations in DNA [3-5], which are often detected in various cancer cells, it is important to determine the levels of 8-oxo-Gua generation in nuclear DNA, when exploring the molecular mechanisms of carcinogenesis (Figure 2A and 2B). In the differentiation of ES cells, DNA damage generated by ROS should be a critical event, because DNA damage-induced mutations in ES cells may result in catastrophic changes that would affect many different cell types in the organism. Therefore, it is reasonable to predict that ES cells should be equipped with highly efficient defense mechanisms against oxidative stresses and oxidative DNA damage [6].

In the context of genomic integrity, ROS generation should be reduced in stem cells. In other words, the defense ability against ROS insult might be gradually weakened during cell differentiation. In fact, several genes encoding antioxidant enzymes, such as thioredoxin-

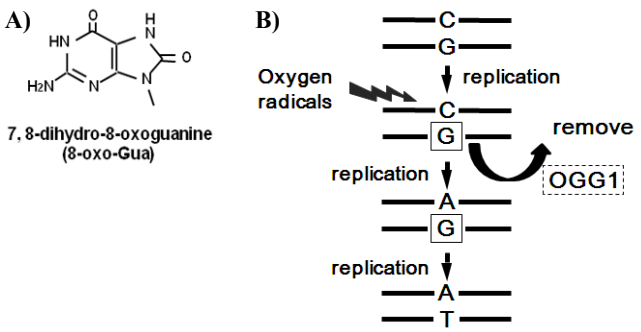

Figure 1: (A) Structure of 7, 8-dihydro-8-oxoguanine (8-oxo-Gua). 8-Oxo-Gua is formed by hydroxylation of guanine at the C-8 position. (B) Mechanism of GC-to-TA point mutation induction. OGG1 is 8-oxoguanine DNA glycosylase, which removes 8-oxo-Gua generated in DNA.

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glutathione reductase, glutathione peroxidases 2, 3, and 4, and glutathione-S-transferase, reportedly became downregulated during the differentiation of mouse ES cells into embryoid bodies [7]. As for 8-oxo-Gua generation, the steady state levels of 8-oxo-Gua were lower in human ES cells than those in differentiated human primary fibroblasts, WI-38 [8]. These studies suggested that the defense capacity for genomic integrity is higher in ES cells than in differentiated cells, and gradually decreases during cell differentiation. Cervantes et al. [9] reported that the mutation frequency in ES cells was significantly lower than that in mouse embryonic fibroblasts. To prevent mutations in genomic DNA, several DNA repair systems are ubiquitously present, from bacteria to human [10,11]. Therefore, the defense capacity against ROS or DNA repair systems is probably higher in ES cells than in differentiated cells.

To assess the protective capacity of ES cells against oxidative DNA damage generation, we previously measured the 8-oxo-Gua generation level and its repair capacity in mouse ES cells and their differentiated counterparts. Our study provided valuable information for the use of stem cells for regenerative medicine in the future. In this short review, we describe oxidative DNA damage and ES cell differentiation, by summarizing our previous work [12] and that of other researchers.

Stem Cells and ROS Generation

Cells constantly suffer from ROS exposure, and thus have defense mechanisms against ROS generation. For stem cells, because oxidative DNA damage-induced mutations in genomic DNA may result in catastrophic changes that would affect many different cell types in the organism, the protective capacity is quite important. Therefore, stem cells should be equipped with more effective antioxidant systems than those of mature differentiated cells, to reduce ROS generation or detoxify ROS. Recent reports indicated that the activities of effective antioxidant defense mechanisms in ES cells diminish during differentiation. These defense mechanisms rely upon the expression of high levels of antioxidant enzymes, such as mitochondrial and cytoplasmic superoxide dismutases, catalase, and peroxiredoxins [7,13,14], and thus mitochondrial superoxide production and cellular levels of ROS increase during differentiation [14]. Yin et al. [15] reported that the amount of reduced GSH was decreased in response to the differentiation of ES cells into vascular smooth muscle cells. These studies suggested that the cell defense capacity to ROS is higher in stem cells than in differentiated cells, and becomes attenuated during cell differentiation. However, the molecular mechanisms underlying the cell defense capacity are still unclear. Quite recently, an interesting study revealed that testicular receptor 4 (TR4), a key transcriptional factor regulating various biological activities, might mediate the interplay between oxidative stress defense and aging [16,17]. Mice lacking TR4 (TR4 (-/-)) exhibited increased genome instability and defective oxidative stress defense [17]. Taken together, as defense systems, ES cells may maintain their genomic integrity by minimizing the levels of ROS, through a combination of enhanced removal capacity and limited production of these molecules.

On the other hand, the production of ROS is also likely to be important in the differentiation or development of stem cells, as regulators of bioactivities. ROS play important roles as second messengers in biological processes. For example, in mechanical strain induced-cardiovascular differentiation, ES cells utilize ROS, generated by the cell membrane-associated NADPH oxidase (Nox) activity, as transducers [18]. ROS signaling cascades are also reportedly involved in cardiomyogenesis induced by exogenous electric stimulation and by prenylflavonoids, such as icariin and icaritin [19,20]. Sauer et al. [21] demonstrated that an incubation with hydrogen peroxide (H_2O_2) enhanced cardiomyogenesis, by activating the Mitogen Activated Protein Kinase (MAPK) pathways in mouse embryoid bodies. Heo and Lee reported the involvement of the β-catenin-mediated signaling pathway in cyclic strain-stimulated cardiomyogenesis, through ROS-dependent and integrin-mediated phosphoinositide 3-kinase (PI3K)-Akt signaling cascades [22]. Furthermore, Lange et al. [23] revealed that the pro-vasculogenic effects of platelet-derived growth factor BB (PDGF-BB) were mediated by Ca^{2+}-induced ROS generation. Besides cardiomyogenesis, the involvement of ROS in the differentiation of other cell types was reported. Ji et al. [24] demonstrated that ROS enhanced the differentiation of human ES cells into the mesendodermal lineage. Another role of ROS in stem cell differentiation was also proposed. ROS suppressed N-cadherin-mediated cell adhesion and induced the exit of hematopoietic stem cells from the niche [25]. Taken together, it is likely that growing stem cells are subjected to mechanical forces generated by ROS, which may initiate differentiation programs. In conclusion, ROS play a critical role in the regulation of the stemness and the differentiation of stem cells.

In addition, it is well known that ROS are generated mainly via a cell membrane-associated Nox or within the respiratory chain of

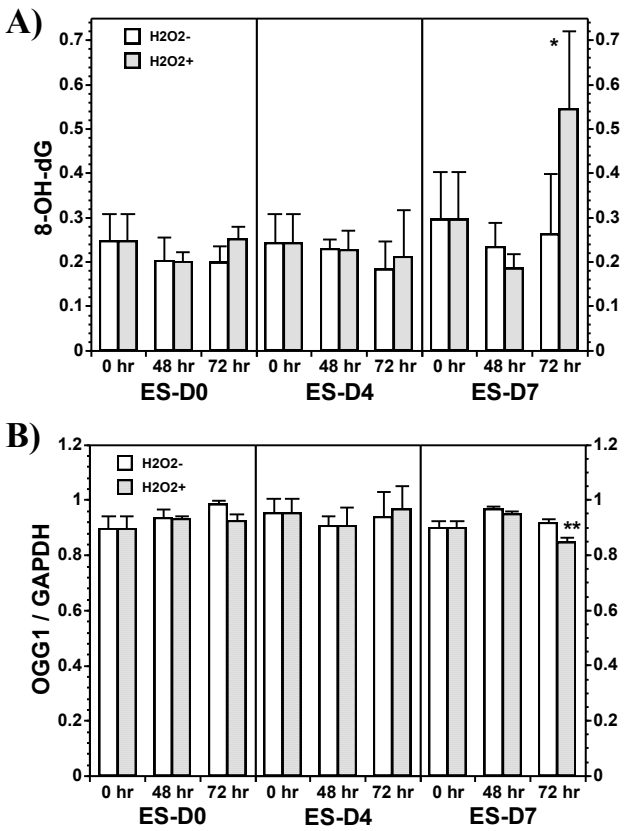

Figure 2: (A) The levels of 8-oxo-Gua generated in the DNA of ES cells. The 8-oxo-Gua levels are expressed as the number of 8-oxo-deoxyguanosine (8-oxo-dG) per 10^5 deoxyguanosine (dG). (B) *Mouse OGG1* mRNA expression. The expression of *mOGG1* mRNA was lower in ES-D7 treated with 300 μM H_2O_2 for 72 hrs, as compared to other cells with / without H_2O_2 treatment. The *mOGG1* expression levels were standardized with the *GAPDH* expression levels. This figure was published in reference [12], Copyright Informa Healthcare.

mitochondria. As for ES cells, since they have only a few mitochondria with immature morphology, ROS have been shown to be predominantly generated via Nox in ES cells [26], suggesting the possibility that the inhibition of mitochondrial function may prevent differentiation, and thus modulate the maintenance of pluripotency [27].

8-Oxoguanine and its Repair Systems

8-Oxo-Gua is a mutagenic lesion formed spontaneously in the genomic DNA of aerobic organisms (Figure 1) and by the actions of exogenous factors, such as ionizing radiation, chemical substances, heavy metals, food, and bacteria [28-33]. Although 8-oxo-Gua is not necessarily the most abundant form of oxidative DNA damage, it has been the most extensively studied, because it can be quantitated with high sensitivity by high performance liquid chromatography coupled with electrochemical detection (HPLC-ECD), and is quite easily measured in laboratories [34,35].

Since 8-oxo-Gua was discovered and reported in 1984 [1], this form of DNA damage and its repair systems have been studied vigorously. 8-Oxo-Gua induces GC-to-TA transversion type point mutations [4], and thus it is believed to play a key role in cancer development. To remove the damage, 8-oxo-Gua repair enzymes exist in a wide variety of bacteria, yeast, mammals, and even plants [6]. In terms of the gene stability associated with DNA damage and its repair system, this fact highlights the primary importance of 8-oxo-Gua and its repair enzyme, 8-oxoguanine DNA glycosylase 1 (OGG1), in most living organisms. 8-Oxo-Gua is efficiently removed from DNA via the short-patch base excision repair (BER) pathway, initiated by OGG1. To control 8-oxo-Gua generation, many organisms possess a three-component enzymatic system (termed the "GO system"), which reduces mutagenesis by 8-oxo-Gua and possibly other oxidized purines [6]. The human GO system consists of three enzymes: OGG1, a homologue of Mut Y (MUTYH), and a homologue of Mut T (MTH1). OGG1 is a major repair enzyme that removes 8-oxo-Gua from DNA (Figure 1B) [36-38]. MUTYH removes adenine from Adenine: 8-oxo-Gua pairs, which only occur in DNA after misincorporation events [39]. MTH1 prevents the incorporation of 8-oxo-Gua into DNA from the pool of oxidized dGTP [40].

Taken together, the level of 8-oxo-Gua measured in DNA depends on the balance between 8-oxo-Gua generation and its repair capacity [6].

Differentiation of ES cells and DNA Damage / DNA Repair Systems

As we mentioned above, the integrity of the genomic DNA in ES cells should be more strictly protected against ROS insults than that in differentiated somatic cells. The protective means by which mutations were suppressed in mouse and human ES cells was reportedly several-fold higher [9,41,42] in comparison to that in somatic cells, suggesting that the DNA repair system should function with a higher capacity in ES cells.

We recently found that the defense capacity against 8-oxo-Gua was also higher in ES cells than in their differentiated counterparts [12]. In the study, we measured the 8-oxo-Gua generation level in the DNA of ES cells and their differentiated counterparts. We used three stages of ES cells, ES-D0, ES-D4, and ES-D7. ES-D0 cells are undifferentiated ES cells. They were cultured with leukemia inhibitory factors (LIF) to maintain the undifferentiated condition. ES-D4 and ES-D7 cells are ES-D0 cells cultured without LIF for 4 and 7 days, respectively. These three cell lines were treated with 300 μM H_2O_2 for 48 and 72 hr (Figure 3). After the H_2O_2 treatment, the amounts of 8-oxo-Gua in the cells were determined by the HPLC-ECD method. The results indicated that the levels of 8-oxo-Gua in ES-D7 treated with H_2O_2 were higher than those in ES-D0 and ES-D4 treated with 300 μM H_2O_2, suggesting that the ES cells were more resistant to the generation of oxidative DNA damage than the differentiated cells. Moreover, similar to our experiment using ES cells, Hildrestrand et al. [43] reported that both the expression and activity of OGG1 were high in neurospheres derived from newborn mice, and were decreased in adults and upon the induction of cell differentiation. These studies indicated that the protective capacity against 8-oxo-Gua is higher in undifferentiated cells, and then decreases during differentiation along with the defense against ROS.

As we mentioned above, the antioxidant cell response is altered during cardiac differentiation and may potentiate the damage from the ROS produced during the differentiation process. The situation is the same for oxidative DNA damage. 8-Oxo-Gua levels were increased and its excision repair capacity was attenuated during the differentiation of skeletal myoblasts into myotubes, suggesting that ROS generation was increased during cell differentiation [44]. Xiao et al. [45] indicated that ES cell differentiation into smooth muscle cells was mediated by Nox 4-produced H_2O_2. The accumulation of oxidative DNA damage, due to attenuated repair capacity, might contribute to myofiber degeneration, as seen in sarcopenia and many muscle disorders. In addition to BER activities such as OGG1 action, nucleotide excision repair (NER) is also strongly attenuated in human cells of the monocytic lineage when they differentiate into macrophages [46].

Although the molecular mechanisms underlying the attenuation of DNA repair capacity during differentiation are still unclear, the roles of p53 as a teratological suppressor have been implicated in the enhanced

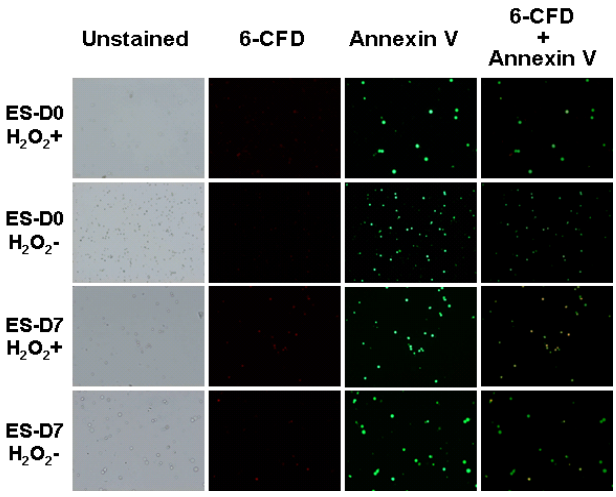

Figure 3: Cells treated with 300 μM H_2O_2 for 72 hrs were examined by the AnnCy3 immunofluorescent method. When cells are incubated with both AnnCy3 and 6-CFDA, living cells and necrotic cells are labeled with 6-CF (green) and AnnCy3 (red), respectively. Cells in the early stage of apoptosis will be labeled with both AnnCy3 (red) and 6-CF (green). Staining with both AnnCy3 (red) and 6-CF (green), which appeared as yellow fluorescence, was frequently observed in ES-D7 cells treated with 300 μM H_2O_2. ES-D0 treated with 300 μM H_2O_2 showed only living cells. This figure was published in reference [12], Copyright Informa Healthcare.

protection of genomic DNA of ES cells, in comparison to differentiated cells [47-50]. Interestingly, a high level of p53 expression was observed in ES cells, and differentiation *in vitro* resulted in a decrease in the level of p53 [51]. In addition, Abdelalim and Tooyama reported that natriuretic peptide receptor-C (NPR-C) was required to control DNA damage-induced p53 levels, to maintain ES cell self-renewal [52].

In general, organisms might seek to protect the small number of valuable slowly dividing stem cells, including ES cells, by augmenting DNA repair [53].

Conclusions

Numerous studies have indicated that undifferentiated cells are more resistant to ROS insults, including ROS-induced DNA damage, in comparison to differentiated cells. There might be at least two reasons why ES cells are equipped with a high defense capacity against ROS insult. One is to protect their genomic DNA from oxidative stresses. Another is to maintain their stemness. However, when excess ROS are produced, and the defense mechanisms in ES cells do not work and the cells cannot use ROS as transducers, then oxidative DNA damage will accumulate. The presence of a high level of p53 will lead them to apoptosis in response to increased levels of oxidative DNA damage.

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