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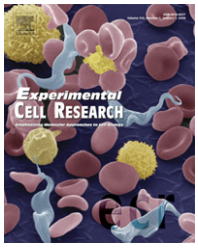

Review

DNA repair in murine embryonic stem cells and differentiated cells

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ABSTRACT

Embryonic stem (ES) cells are rapidly proliferating, self-renewing cells that have the capacity to differentiate into all three germ layers to form the embryo proper. Since these cells are critical for embryo formation, they must have robust prophylactic mechanisms to ensure that their genomic integrity is preserved. Indeed, several studies have suggested that ES cells are hypersensitive to DNA damaging agents and readily undergo apoptosis to eliminate damaged cells from the population. Other evidence suggests that DNA damage can cause premature differentiation in these cells. Several laboratories have also begun to investigate the role of DNA repair in the maintenance of ES cell genomic integrity. It does appear that ES cells differ in their capacity to repair damaged DNA compared to differentiated cells. This minireview focuses on repair mechanisms ES cells may use to help preserve genomic integrity and compares available data regarding these mechanisms with those utilized by differentiated cells.

Contents

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Mutation frequencies in embryonic stem cells and differentiated cells

Embryonic stem (ES) cells are derived from cells of the blastocyst inner cell mass, are pluripotent, and have virtually unlimited self-renewal potential. Upon differentiation, these cells can contribute to the formation of cells of all types within an organism, including germ cells. To prevent an undue mutational burden at the level of both the individual and the species, it is important that they have robust mechanisms to ensure their genomic integrity. Mutations in differentiated somatic cells can lead to somatic diseases involving those cell lineages and cells with which they interact. In contrast, mutations in ES cells can result in catastrophic changes that can affect many different cell types in the organism and that may be passed on to its progeny.

There are distinct differences in mutation frequencies between ES cells and somatic cells, and hence in the maintenance of genomic integrity [1]. When the selectable *Aprt* gene is used as a reporter for mutagenesis in ES cells heterozygous at *Aprt*, they display spontaneous mutation frequencies that approach 10^{-6}. While this mutation frequency is somewhat lower than estimates using other model systems [2,3], ES cells display substantially lower frequencies than those in somatic cells. Mouse embryo fibroblasts (MEFs), for example, have mutation frequencies nearly 100-fold greater ($\sim 10^{-4}$) than their isogenic ES cell counterparts, and most of these events involve loss of heterozygosity (LOH) due to mitotic recombination. When spontaneous mutation frequencies at the *Hprt* gene were assessed in similar manner in ES cells and somatic cells, spontaneous mutation in ES cells was undetectable ($<10^{-8}$) whereas mutation frequency in MEFs was in the range of 10^{-5}. The *Hprt* gene is X-linked and therefore not susceptible to LOH as a consequence of mitotic recombination, which probably accounts for much of the difference in mutation frequency between *Aprt* and *Hprt*. In both cases, there was a dose-dependent elevation in mutation frequency when ES cells were exposed to ethyl methanesulfonate (EMS), a mutagenic alkylating agent. These data are consistent with the contention that ES cells have robust mechanisms to ensure the preservation of genetic stability. Spontaneous mutation in either cell type was generally the result of LOH at the *Aprt* locus (80%) vs. point mutation (20%); however, the spectrum of LOH induced mutations was very different between ES cells and MEFs. Whereas MEFs displayed mainly mitotic recombination to generate LOH, ES cells exhibited mainly nondisjunction, and to a lesser extent, mitotic recombination [1]. An independent study investigating LOH in ES cells reported a similar spectrum of events in ES cells [4].

While suppression of mutagenesis in ES cells appears to be one of the mechanisms that contribute to preservation of genomic integrity, it is not, by itself, sufficient. ES cells are hypersensitive to DNA damage and readily undergo apoptosis or differentiation which removes damaged cells from the pluripotent pool [5,6]. Loss of damaged self-renewing cells effectively maintains the proliferating cell population genetically pristine. Consistent with this observation, ES cells lack a functional G1 checkpoint, partly due to sequestration of p53 in the cytoplasm. A possible consequence of the absence of a G1 arrest is that cells with DNA damage can transit from G1 into S-phase where the damage can be exacerbated by proceeding through a round of replication [7–9]. Recently, it was reported that p53 facilitates differentiation by translocating to the nucleus and associating with the Nanog promoter and inhibiting its transcription, suggesting that the role of p53 is more important during differentiation than in responding to DNA damage in ES cells [10]. By supporting ES cell differentiation and consequent withdrawal of cells from the self-renewing population, this mechanism also helps maintain a pure population of cells. Several studies currently focus on the role that DNA repair plays in maintaining genomic stability in ES cells. Few studies, however, specifically compare the repair capacities between ES cells and somatic cells. The remainder of this review focuses on DNA repair in ES cells, and compares these processes to those of somatic cells when data for such comparisons exist.

Double-strand break repair

Double strand breaks (DSBs) in DNA are the most toxic type of DNA lesion a cell encounters [11]. Repair of DSBs is expected to be important for ES cells, since there is a high basal level of γ-H2AX staining, a common marker of DSBs (Fig. 1). In contrast, unchallenged MEFs display no detectable staining with γ-H2AX. Treatment with etoposide, a topoisomerase II poison that generates DSBs, markedly increases γ-H2AX staining in both cell types. The possible causes of the high level of basal staining in ES cells could be the result of replication fork collapse or reactive oxygen species (ROS) from oxidative metabolism. The latter, however, is unlikely since Saretzki et al. [12] demonstrated that ES cells can be grown in hyperoxic conditions (40% O_2) with little effect on cell proliferation compared with cells grown under normoxic culture conditions. When MEFs were grown in hyperoxic conditions, they underwent fewer than half the number of population doublings compared with those grown in normoxic conditions. This study also suggests that ES cells repair DSBs far more quickly than mouse 3T3 cells following exposure to IR. Which type of DSB repair occurs, however, was not addressed [12].

There are two major pathways for DNA DSB repair. These are: homologous recombination-mediated repair (HRR) and nonhomologous end-joining (NHEJ). In HRR, repair of DSBs involves the use of a template containing hundreds of base pairs of sequence homology, usually the sister chromatid or homologous chromosome, resulting in faithful, error-free repair. This pathway is active predominantly in the late S to G2 phases of the cell cycle, where sister chromatids are available to serve as templates [13–15]. Many of the proteins involved in this pathway belong to the RAD52 epistasis group and are conserved from yeast to mammals [16]. Mice in which a subset of genes involved in HRR has been knocked out result in early embryonic lethality, suggesting a critical role for these genes in early development [17]. The other major DSB repair pathway is NHEJ which requires little or no sequence homology for efficient repair and can be error-free or error-prone, depending on the type of ends present at the site of the DSB. Direct religation of compatible ends results in error-free repair. If end processing is required prior to religation, however, nucleotides can be deleted or added at the site of the repaired lesion. In contrast to HRR, NHEJ occurs more commonly in the G1 and early S phases of the cell cycle, when a sister chromatid is not available to serve as a template for repair.