The role of methyltransferase DNMT2 in cellular stress responses and the regulation of cellular senescence in mammalian fibroblasts

SUMMARY

In the last few years, many research studies have documented direct evidences of correlation between the highest incidence of age-related diseases and cellular senescence. In addition, it has been shown that cellular senescence plays a role in cancer. Understanding the molecular mechanisms and causes that promote cellular senescence can help to reduce the risk of developing age-related diseases. Thus, identification of novel factors and the development of new therapeutic approaches that might modulate cellular senescence are important and DNMT2 would be a potential candidate for this purpose.

DNMT2 is a member of the DNA methyltransferases family of enzymes. It has been suggested that DNMT2 may be involved in both tRNA methylation and DNA methylation. As dDnmt2 may be implicated in the regulation of lifespan in the fly *Drosophila melanogaster* and such regulation may be associated with oxidative stress resistance, the purpose of the present study was to examine the role of DNMT2 methyltransferase in cellular stress responses and in the regulation of mammalian fibroblasts senescence *in vitro*.

The main goal of this work was to study the effects of DNMT2 silencing on senescence of mammalian fibroblasts *in vitro* during two experimental conditions, namely in the control conditions as well as in hydrogen peroxide-induced stress conditions. The experiments were performed using three commercially available cell lines, namely NIH3T3, WI-38 and BJ. Changes in cell cycle, cellular senescence, proliferation, apoptosis, telomere length, telomerase activity, production of reactive oxygen species, protein carbonylation, DNA damage and epigenetic modifications were investigated. The obtained results provide a comprehensive characteristics of the phenotype of DNMT2-depleted fibroblasts that allows for a comparison with non-genetically modified cells.

The study showed that the DNMT2 methyltransferase may be a modulator of mammalian fibroblasts senescence as well as is involved in cellular response to hydrogen peroxide-induced stress. Furthermore, CRISPR-based stable knockdown of *Dnmt2* in NIH3T3 mouse fibroblasts induced cellular senescence in normal laboratory conditions and stress conditions, which was accompanied by decreased telomere length and telomerase activity, protein carbonylation, redox imbalance, DNA damage and increased expression of cell cycle inhibitors, namely p53 and p21. Interestingly, Dnmt2-depleted cells have been found to be more sensitive to hydrogen peroxide-mediated protein carbonylation.

Transient siRNA-based DNMT2 silencing induced cellular senescence and apoptotic cell death in WI-38 and BJ normal human fibroblasts, which was appeared to be induced by dysregulation of intracellular redox homeostasis (increased production of reactive oxygen species and protein carbonylation), genetic instability, elevated levels of p16, p21 and p53 that resulted in the inhibition of cell proliferation. Moreover, *DNMT2*-depleted human fibroblasts were more sensitive to hydrogen peroxide-induced apoptosis compared to control cells (non- transfected and transfected siRNA-FITC). Additionally, the cellular levels of DNMT2 were overexpressed in replicatively senescent human fibroblasts and protein localized in the cytoplasm. In control siRNA transfected cells elevated levels of DNMT2 was also observed.

Based on current knowledge, the results presented in this study are the first report on mouse Dnmt2 and human DNMT2- mediated effects on lifespan, apoptosis, cell proliferation and cellular stress responses in mammalian fibroblasts. Therefore, it can be concluded and postulated that the manipulation of DNMT2 methyltransferase levels that induce telomere dysfunction, cause a decrease of telomerase activity, and inhibition of cell proliferation and apoptosis in senescent as well as damaged cells could be a potential novel anti-aging (senolytic activity) and anticancer strategy. However, such assumption requires further investigation and evidence *in vivo* to be fully confirmed.

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