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SIRT1 regulates autophagy and diploidization in parthenogenetic haploid embryonic stem cells

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ABSTRACT

Recent studies have demonstrated that parthenogenetic haploid embryonic stem cells (designated as ph ESCs) was difficult to maintain the haploid status or cell viability over time during differentiation or high passages, as undergoing readily self-diploidize spontaneously. Here, we found that age-related oxidative stress and autophagic cell death in ph ESCs at high passage were close to 1 mM H_2O_2 treatment. Exogenous H_2O_2 tended to promote SIRT1 expression and induce more autophagy through mTOR pathway in control ph ESCs, by contrast more apoptosis via activation of p53 and caspase-3 in SIRT1-knockdown ph ESCs. Furthermore, we also evaluated that SIRT1 directly decreased p53 expression via increasing H3K9 di- and tri-methylation in both nucleus and cytoplasm of ph ESCs, whereas indirectly inhibited DNA demethylation and replication through H3K9me2 blocking TET3. In summary, the results revealed that the diploidization of ph ESCs at high passage might correlate with SIRT1 as an important role in regulating autophagy and TET3 expression.

1. Introduction

Haploid embryonic stem cells (hESCs) are a group of cells containing only one set of chromosome and resembling characteristic with normal embryonic stem cells (ESCs), and giving rise to a wide range of functioning cell of all tissues and organs [1]. Since 2011, several research groups have independently established haploid embryonic stem cells (hESCs) successfully from mouse haploid parthenogenetic or androgenetic embryos [2,3]. Abundant data show that hESCs can offer new possibilities for genetic screening analyses, further produce genetic models for recessive traits, development and disease studies *in vitro* or *in vivo* [4,5].

However, the haploid state of hESCs frequently makes conversion into spontaneous diploid status during differentiation or high passages [6]. Diploid status makes mutation screens more complicated and limits efforts to identify the function of specific genes. The mechanism for readily diploidizing of hESCs remains unclear now, but the age-related pathologies might result from cellular stress conditions during high passage [7]. In certain situations, autophagy has often been regarded as a defense mechanism to protect cells under stress conditions [8], but it has been complicated by the identification that whether autophagy or apoptosis mainly occur in diploidizated hESCs at high passage.

Here, we generate parthenogenetic haploid embryonic stem cells (ph ESCs) with the goal of evaluating connections between induction of autophagy and spontaneous diploidization in haploid ESCs. As known, the lifespan extension capability of the mammalian silent information regulator (SIRT1) is associated with the mechanism responding to stress condition involving deacetylation of key prosurvival molecule regions such as H3K9 *in vivo* [8–10]. Based on previous reporter, SIRT1 contributes to the release of age-related reactive oxygen species (ROS) induced by high concentrations of H_2O_2 (1 mM), which is highly dependent on mitochondria metabolism, ultimately leading to the induction of apoptosis and autophagy [9]. So we evaluated connections between SIRT1 activity and autophagy in ph ESCs upon oxidative stress.

2. Materials and methods

2.1. Derivation of ph ES cells

Cumulus-free MII phase oocytes (C57BL/6 background) were collected and incubated in 5 mM $SrCl_2$ for 6 h in a 37 °C incubator to generate parthenogenetic haploid embryos and subsequently were plated into M16 medium drops for further culture. Single haploid

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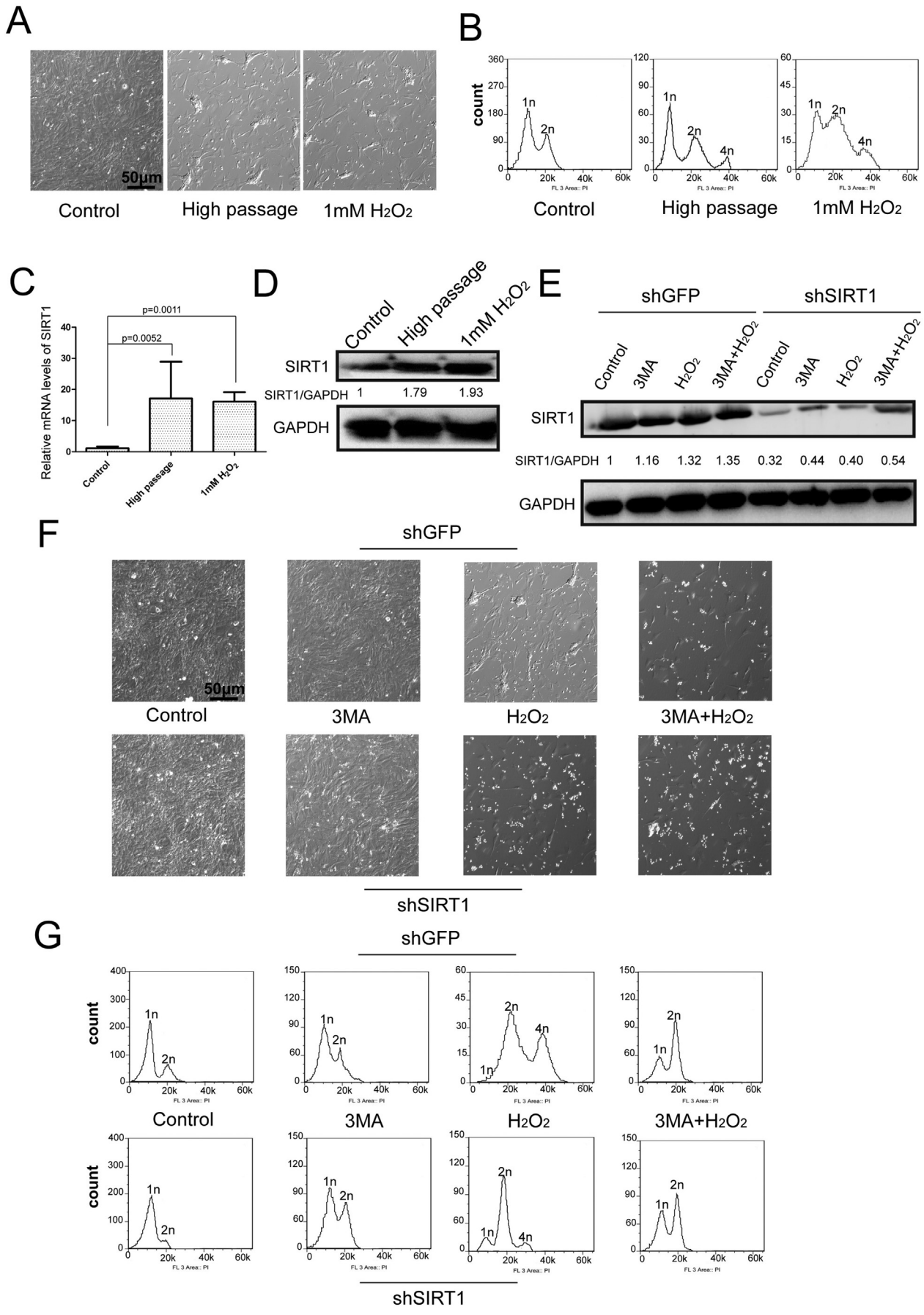

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