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Effects of cryopreservation on the epigenetic profile of cells

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ABSTRACT

Effective cryopreservation protocols are essential for long-term storage of cells and their subsequent clinical application. Freezing protocols are generally considered as safe; however, putative effects on epigenetic marks have not yet been studied in detail. While post-thaw cell survival rates have been used to evaluate the success of cryopreservation protocols, increasing evidence suggests that freezing may be associated with deviations from the physiological epigenetic marks with putative long-term effects on the cells and/or their derivatives. A better understanding of the underlying mechanisms would be beneficial for improving safety and effectiveness of freezing protocols. The purpose of this review is to provide current information regarding epigenetic alterations (DNA methylation and histone modification patterns) associated with cryopreservation.

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1. Introduction

Cryopreservation allows long-term preservation of viable cells and tissues at ultra-low temperatures in a state of suspended animation. It has major impacts on reproductive medicine, animal husbandry and conservation of endangered species [64]. Cryopreservation protocols essentially include two methods: conventional slow freezing and ice-free cryopreservation or vitrification. Conventional slow freezing usually implies cooling samples at rates of 1–2 °C/min followed by rapid thawing. Ice-free cryopreservation is a cooling process during which a solution rapidly solidifies without the formation and growth of ice crystals. This can be performed by ultra-rapid cooling of the solution in combination with a high solute concentration, such that the increased viscosity prevents nucleation and growth of ice crystals, stopping molecular motion, and ultimately causing the solution to enter a “glassy-

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state" [38]. Both methods are effective and have their specific advantages as well as shortcomings. Slow freezing may damage cells mainly by intracellular ice formation and solution effects, whereas elevated levels of cryoprotectants, osmotic damage and small sample volume are major disadvantages of ice-free cryopreservation [38].

1.1. Cryovariables

An overview of cryovariables, including cooling and thawing rates, type and concentration of the cryoprotectant, cell type and shape, and nucleation temperature that may affect the success of cryopreservation has been illustrated in Fig. 1.

Studies have shown that the optimum cooling rate may differ for different cell types [33,72]. The probability of intracellular ice formation is dependent on the cooling rate, in line with the two-factor hypothesis of freezing injury [1]. Cryoprotective agents (CPAs) are added to the freezing medium to protect cells from injury during the freezing and thawing processes. The presence of CPAs in freezing media leads to a higher post-thaw survival rate by protecting the cells from cryopreservation induced stress [45]. CPAs can be divided into two categories: penetrating and non-penetrating cryoprotectants. The penetrating diffusible CPAs such as dimethylsulfoxide (DMSO) and glycerol usually have a molecular weight smaller than 400 Da and can passively cross cell membranes. Non-penetrating CPAs are non-diffusible and remain in the extracellular solution and thus cannot cross the cell membrane because of higher molecular weight; examples are hydroxyethyl-starch (HES) or poly[*N*-vinyl-2-pyrollidone] (PVP) [45]. However, CPAs can also lead to toxicity in a time and temperature dependent manner. Attempts have been made to minimize or even fully prevent toxic effects of CPAs by reducing the exposure prior to and after freezing and using lower temperatures for exposure [20]. Different solutes have been investigated with regard to efficiency to act as CPAs, including alcohols, diols, amides, sugars and amino acids [19,32,76]. Survival after cryopreservation also depends on the type and shape of cells and their permeability to various CPAs. In case of human sperms, it is known that addition and removal of glycerol during cryopreservation may result in cellular injury due to osmotic stress [22]. Each cell type requires optimized conditions for successful cryopreservation. Thus, for each batch of cells the optimal freezing protocol has to be determined taking specific cellular properties into account. This scenario becomes even more complex for multi-cellular systems such as tissues and organs that consist of a heterogeneous population of different cell types. Higher concentrations of CPAs ensure an overlapping of survival curves of many cell types, resulting in a more flattened bell shaped curve with broader peaks [63]. The nucleation temperature is another important cryovariable. When uncontrolled, the nucleation temperature significantly affects cell recovery, viability and function which ultimately lead to high variability between samples. Samples with very small volumes, including stem cells, hepatocytes, peripheral blood mononuclear cells, fungi, protozoa, yeast and bacteria, have shown higher survival rates after use of controlled ice nucleation [20,21,29,74]. Storage temperatures have also been shown to influence cryopreservation outcome of various biological specimens [37].

1.2. Applications of cryopreservation

Cryopreservation has significant impact on stem cell-based therapies, assisted reproductive technology (ART), germplasm conservation and plant biotechnology. Hematopoietic stem cells and mesenchymal stem/stromal cells have been used for treating hematological and non-hematological diseases. For commercial use of stem cells, well established and reliable cryopreservation protocols become mandatory to meet the standards of the regulatory bodies [38]. Freezing of human sperm and oocytes is now a routine procedure in ART programs. ART is useful for patients suffering from infertility caused by cancer treatment and/or chronic diseases. Higher cumulative conception rates were achieved with couples undergoing ART following one cycle of ovarian stimulation, followed by IVF and the use of advanced techniques for freezing of zygotes and embryos [51]. In animals, freezing of germplasm involves the preservation of oocytes, sperm and embryos of a wide variety of domestic animal species, including cattle, horse, sheep, aquatic species and is widely applied in breeding programs. The establishment of germplasm banks has significantly contributed to protecting rare and endangered species from extinction [79]. Moreover, cryopreservation of valuable plant genes, embryogenic tissues, protoplasts and transgenic plants has contributed to an increased production of food crops to cope with the challenges

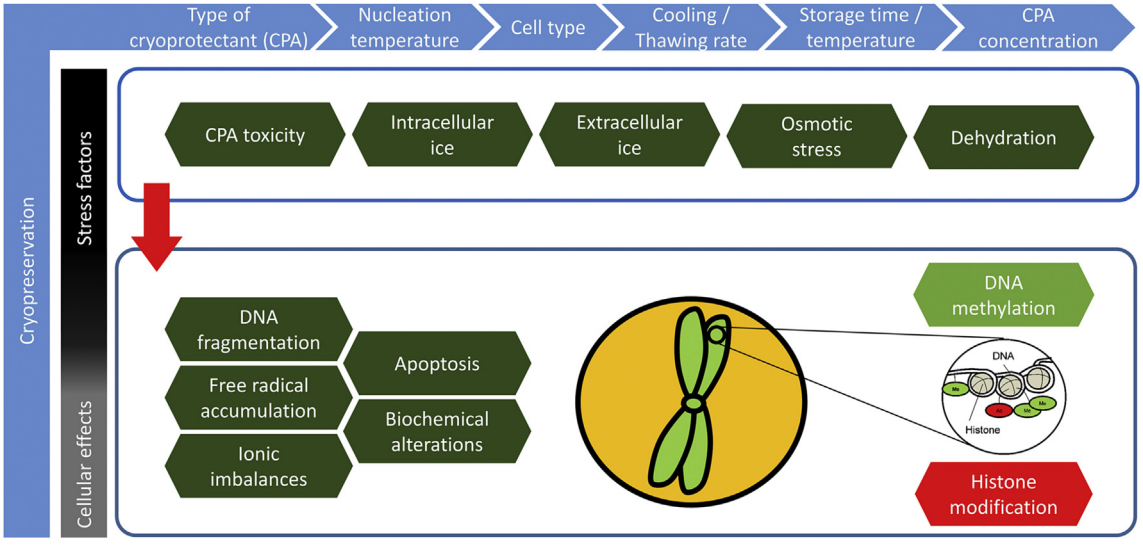

Fig. 1. Cellular effects of cryopreservation. Cellular damage occurring during cryopreservation may be attributed to epigenetic alterations associated with suboptimal cryovariables used for cryopreservation.