Limbal Stem Cell Senescence Cultivated on Human Amniotic Membrane by Explant Culture Method

Background:

Limbal stem cell deficiency is a pathological state marked by corneal neovascularization and conjunctivalization. In this disease, deficient limbus may not be able to sustain the turnover and proliferation of cornea epithelial cells and prevent invasion of conjunctival epithelium, resulting in inevitable visual loss. Recently, limbal stem cell (LSC) transplantation has attracted enormous interest as a therapeutic method which mainly involves in-vitro expansion of LSCs. However, it is believed that ex-vivo cultivation conditions could impose oxidative stress on the cells, leading to a decline in their proliferative and regenerative potency followed by a premature-stress induced senescence. An optimal potency of stemness is necessary for a successful transplantation which could be achieved by optimizing expansion conditions for LSCs. Considering these facts, we conducted a survey on ex-vivo culture-expanded LSCs in order to delay their senescence during the culture process by using N-acetyl-cysteine as a well-known antioxidant agent.

Method:

The presence of cells in limbus area was assessed by hematoxylin and eosin staining. LSCs were obtained from human corneal rims and cultivated in three groups: the test group, treated with N-acetyl-cysteine; the positive control group, treated with H2O2; and the control group, without any additives. The presence of cells on amniotic membrane after tissue embedding and sectioning procedure was assessed by hematoxylin and eosin staining. Expression of LSC marker, p63, was determined by immunostaining assays. The effect of N-acetyl-cysteine, as an antioxidant, on senescence process of expanded LSCs was evaluated by measuring senescence-associated beta-galactosidase activity.

Results:

The removal of conjunctiva and the presence of cells in limbus area were proved by hematoxylin and eosin staining. Expression of LSC marker, p63, was proved by immunostaining assays. The treated cells with N-acetyl-cysteine both in short treatment group (p-value< 0.0001) and long treatment (p-value= 0.0449) significantly presented low levels of senescence-associated beta-galactosidase as compared with the control group, highlighting the role of N-acetyl-cysteine in delaying the senescence of cultured LSCs.

Conclusion:

Treating LSCs with N-acetyl-cysteine during the ex-vivo cell culture could be effective in delaying the senescence of LSCs during ex-vivo expansion process which could be valuable for long-term cornea restoration.

Keywords: Limbus, Stem cells, Senescence, Oxidative stress