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Proliferative capacity of corneal endothelial cells

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ABSTRACT

The corneal endothelial monolayer helps maintain corneal transparency through its barrier and ionic "pump" functions. This transparency function can become compromised, resulting in a critical loss in endothelial cell density (ECD), corneal edema, bullous keratopathy, and loss of visual acuity. Although penetrating keratoplasty and various forms of endothelial keratoplasty are capable of restoring corneal clarity, they can also have complications requiring re-grafting or other treatments. With the increasing worldwide shortage of donor corneas to be used for keratoplasty, there is a greater need to find new therapies to restore corneal clarity that is lost due to endothelial dysfunction. As a result, researchers have been exploring alternative approaches that could result in the *in vivo* induction of transient corneal endothelial cell division or the *in vitro* expansion of healthy endothelial cells for corneal bioengineering as treatments to increase ECD and restore visual acuity. This review presents current information regarding the ability of human corneal endothelial cells (HCEC) to divide as a basis for the development of new therapies. Information will be presented on the positive and negative regulation of the cell cycle as background for the studies to be discussed. Results of studies exploring the proliferative capacity of HCEC will be presented and specific conditions that affect the ability of HCEC to divide will be discussed. Methods that have been tested to induce transient proliferation of HCEC will also be presented. This review will discuss the effect of donor age and endothelial topography on relative proliferative capacity of HCEC, as well as explore the role of nuclear oxidative DNA damage in decreasing the relative proliferative capacity of HCEC. Finally, potential new research directions will be discussed that could take advantage of and/or improve the proliferative capacity of these physiologically important cells in order to develop new treatments to restore corneal clarity.

1. Introduction

The corneal endothelium helps maintain corneal transparency via its barrier and ionic "pump" functions. To maintain transparency, endothelial cell density (ECD) must remain above a critical number—usually 400–500 cells/mm^2. Morphometric analyses of ECD in fetal and adult endothelium (Murphy et al., 1984; Bourne et al., 1997; Hollingsworth et al., 2001) indicate that, following formation of the endothelial monolayer during corneal development, human corneal endothelial cells (HCEC) do not normally divide *in vivo* at a rate sufficient to replace dead or injured cells. This results in an average cell loss of 0.3–0.6% per year. The response of the endothelium to this gradual cell loss, as well as to larger wounds, normally involves spreading and/or migration of neighboring cells to cover the wound area (Laing et al., 1976; Honda et al., 1982; Matsuda et al., 1985). The result of this form of wound healing is an increase in overall cell size and an alteration from a hexagonal to a pleomorphic shape. Unfortunately, ECD can be significantly decreased as the result of accidental or surgical trauma, refractive surgery, previous penetrating or endothelial keratoplasty, stress caused by certain diseases such as diabetes or glaucoma, or endothelial dystrophies. If the density of endothelial cells is too low, barrier function is lost and more fluid enters the cornea than can be removed through the activity of the ionic "pumps". Loss of endothelial barrier function results in corneal edema, development of bullous keratopathy, and loss of visual acuity. Current treatments, such as penetrating or endothelial keratoplasty to restore visual acuity generally work well, but can have complications requiring re-grafting or other treatments (Rahman et al., 2010; Lass et al., 2010; Terry et al., 2008; Clements et al., 2011; Shulman et al., 2009). In addition, there is an increasing worldwide shortage of donor corneas that are considered acceptable for transplant purposes and the aging of the "baby boomer" generation will bring a greater need to find new therapies to restore corneal clarity that is lost due to endothelial dysfunction. One approach to develop new therapies to prevent or treat excessive corneal endothelial cell loss

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is to explore the relative ability of HCEC to divide. This review will present information regarding the positive and negative regulation of the cell cycle and discuss results of studies exploring the proliferative capacity of HCEC.

2. The cell cycle

Fig. 1 presents a simplified diagram of the positive and negative regulation of the cell cycle. Additional information related to the cell cycle, but not emphasized here, can be found in recent reviews (Ozaki and Nakagawara, 2011; Kim et al., 2011; Ma and Poon, 2011; and Rieder, 2011). Non-dividing cells normally exist in a "resting" (G_0) state in which DNA is present in an unduplicated (2N) form. Mitogenic stimulation induces G_1-phase entry, which prepares cells for DNA duplication in S-phase. Movement of cells from G_1- into S-phase is highly regulated and involves control of the activity of the E2F transcription factor, which activates genes required for DNA synthesis (Leone et al., 1999). In quiescent cells, E2F is tightly associated with the retinoblastoma tumor suppressor, pRb, which prevents its activation. To inhibit E2F activity, pRb must be in a hypo-phosphorylated state. Hypo-phosphorylation of pRb is maintained, in part, by the activity of the cyclin-dependent kinase inhibitors (CKIs), p27Kip1, p21Cip1, and p16INK4a. Mitogenic stimulation induces a reduction in the protein level of these CKIs due to transcriptional inhibition and/or to increased degradation by the ubiquitin–proteasome pathway. Mitogenic stimulation also induces synthesis of the positive G_1-phase regulatory protein, cyclin D (Sherr, 1993), which binds to cyclin-dependent kinase (CDK)-4, forming an active kinase complex that specifically phosphorylates pRb. This hyper-phosphorylation alters the interaction of pRb with E2F, promoting activation of E2F and leading to S-phase entry. Cyclin E is synthesized late in G_1-phase upon E2F activation. Cyclin E binding to CDK2 helps activate this kinase complex and, in part, promotes continued hyper-phosphorylation of pRb and movement into S-phase. In S-phase, DNA is duplicated under highly controlled conditions, moving DNA from the 2N to a 4N state. Cyclin A synthesis begins in late G_1-phase. Cyclin A binds to CDK2 and the activity of this complex down-regulates E2F activity by facilitating its degradation, thus promoting forward progression from S- to G_2-phase. Cyclin B synthesis is activated at the end of S-phase. In G_2-phase, cyclin B binds to and activates the kinase activity of CDK1, which prepares the cell for M-phase (mitosis), in which cells divide, forming daughter cells, each of which contains 2N DNA.

Negative regulation of G_1-phase involves inhibition of the kinase activity of the G_1-phase cyclin/CDK complexes by CKIs. There are two CKI families. The "INK" family includes p16INK4a, which specifically binds to free CDK4 and prevents its association with cyclin D to form an active complex (Serrano et al., 1993). p16INK4a also competes with cyclin D for binding to CDK4 in existing complexes, thus dissociating the complex. Inhibition of cyclin D/CDK4 kinase activity by p16INK4a prevents the initial downstream hyper-phosphorylation of pRb that is required for E2F activation and S-phase entry. The "Cip/Kip" family includes p21Cip1 and p27Kip1 (Harper et al., 1993; Polyak et al., 1994). Both these inhibitors bind G_1-phase cyclin/CDK complexes, inhibiting their kinase activity. In the presence of p16INK4a, the "Cip/Kip" proteins mainly bind and inhibit the activity of cyclin E/CDK2 complexes. Synthesis of p21Cip1 is induced by the transcription factor, p53, which can be activated by a number of factors, including oxidative DNA damage (Helton and Chen, 2007). Transforming growth factor-β (TGF-β) and formation of mature cell–cell contacts (Polyak et al., 1994) increase the cellular level of p27Kip1. The inhibitory function of all the G_1-phase CKIs is extremely important, because it prevents unscheduled entry into S-phase and inappropriate DNA synthesis.

3. Cell cycle status of HCEC *in vivo*

To understand why HCEC do not proliferate *in vivo*, this laboratory conducted studies to determine the cell cycle status of endothelial cells in *ex vivo* donor human corneas (Joyce et al., 1996a, 1996b). This was accomplished by observing the relative staining intensity and subcellular localization of a battery of key cell cycle

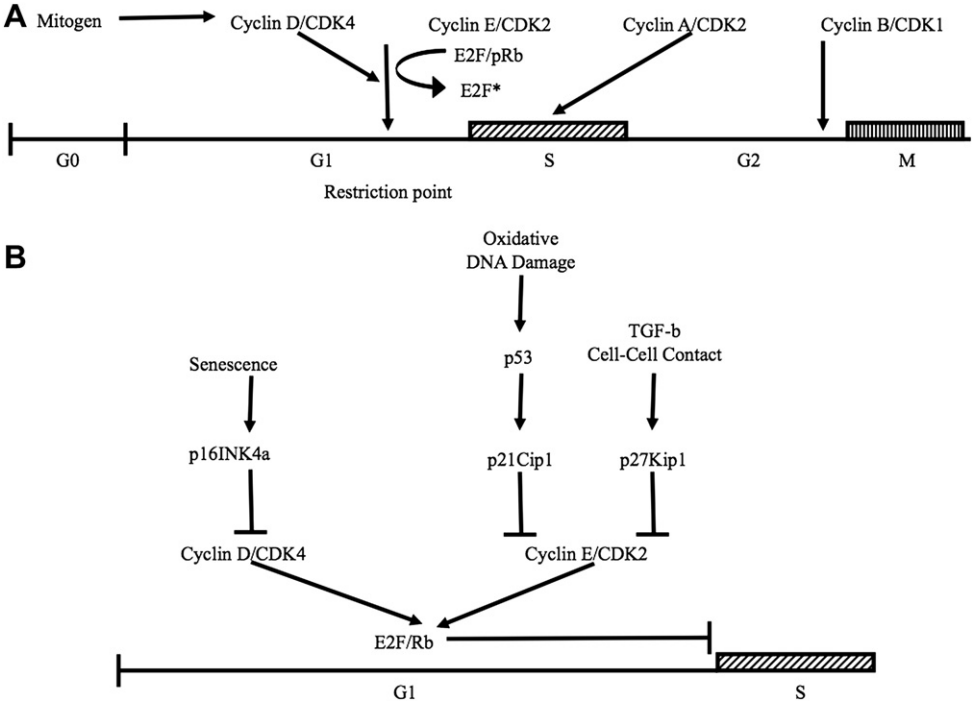

Fig. 1. Diagrams illustrating the positive (A) and negative (B) regulation of the cell cycle. Springer-Verlag is the original copyright holder.