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The role of protein tyrosine phosphorylation in the cell–cell interactions, junctional permeability and cell cycle control in post-confluent bovine corneal endothelial cells

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Abstract

Cell–cell interaction, junctional permeability and contact inhibition are important mechanisms that allow corneal endothelial cells to maintain stable corneal hydration status and also keep these cells in non-proliferative status. Protein tyrosine phosphatases (PTPs) are well known to play an important role in regulating cell–cell contacts, growth and differentiation. Inhibition of PTPs activity by a general PTP inhibitor has been proved to trigger post-confluent rat corneal endothelial cells to reenter cell cycles. In this study, we aimed to evaluate whether protein tyrosine phosphorylation is involved in cell–cell interactions, junctional permeability and cell cycle control in post-confluent, contact inhibited bovine corneal endothelial cells. Confluent cultures of bovine corneal endothelial cells were treated with different concentrations of general phosphatase inhibitor, sodium orthovanadate (vanadate) for several different durations. Protein tyrosine phosphorylation was confirmed by Western blot analysis. Immunocytochemistry was used to evaluate the effect of vanadate on adherens-type junctional proteins by staining of p120, N-cadherin and α-catenin. Paracelluar permeability was evaluated by transepithelial electric resistance. The effect of vanadate on cell cycle progression was confirmed by immunostaining of Ki67. Western blot analysis was used to evaluate the expression level of cell-cycle-associated proteins, including PCNA, cyclin D1, cyclin E and cyclin A. Time-dependent effects of vanadate on protein tyrosine phosphorylation were confirmed by Western blot analysis. ICC demonstrated the effect of vanadate on the disruption of p120, N-cadherin and α-catenin. Time- and dose-effects of vanadate on the severity of p120 disruption were also found. TER demonstrated the time- and dose-effect of vanadte on paracellular permeability. Although cell–cell junctions can be broken through by vanadate, no significant increase of Ki67 positive cells was found among the control group and all groups with different concentrations/durations of vanadate treatment. Western blot also showed no change of PCNA, cyclin D1, cyclin E and cyclin A after treatment with vanadate. In conclusion, protein tyrosine phosphatase inhibition can induce time-dependent release of cell–cell contacts and increase transepithelial permeability in post-confluent cultures of bovine corneal endothelial cells. However, such phenomenon is not enough to promoted cell cycle progression as seen in rat corneal endothelial cells.

Keywords: protein tyrosine phosphatase; vanadate; contact inhibition; trans-epithelial electric resistance; corneal endothelial cells; adherences junctions; p120; proliferation

1. Introduction

Corneal endothelium is a single layer of neural crest-derived cells located on the posterior surface of the cornea. The endothelium helps maintain corneal transparency by regulating corneal hydration through its barrier (Iwamoto and

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Smelser, 1965; Kreutziger, 1976) and ionic pump functions (Maurice, 1972; Geroski and Edelhauser, 1984). Adult human corneal endothelial cells are known to be mitotically inactive, and several studies demonstrate that these cells are arrested at the G1 phase of the cell cycle. (Joyce et al., 1996; Senoo and Joyce, 2000; Senoo et al., 2000) Several mechanisms have been suggested to participate in the non-proliferative status of adult corneal endothelial cells. Among all, cell–cell contact inhibition is an important one that has been widely studied (Joyce et al., 1998; Senoo and Joyce, 2000; Senoo et al., 2000; Joyce, 2003).

In most epithelial cells of various tissues, the cell–cell contact or so called cellular barrier is provided by a combination of the impermeability of the cellular plasma membrane and the intercellular tight junction found towards the apical surface of the cell (Gumbiner, 1987; Anderson et al., 1993; Citi, 1993). The cellular barrier also creates a boundary between the apical and basolateral domains of the plasma membrane and is important in the maintenance of cell surface polarity (Rodriguez-Boulan and Nelson, 1989). Corneal endothelial cells are different from most epithelial cells in the body because of their ''leaky'' tight junctions. Focal, rather than belt-like, tight junctions are located toward the apical aspect of the lateral membranes of corneal endothelial cells (Hirsch et al., 1977; Montcourrier and Hirsch, 1985; Stiemke et al, 1991). These leaky tight junctions are important because they permit diffusion of nutrients from the anterior chamber to the avascular corneal stroma (Mandell et al., 2006). So far, corneal endothelial cells are found to have the following junctional proteins: (1) tight junction-associated proteins, which included occludin (Stevenson et al., 1986; Furuse et al., 1993; McCarthy et al., 1996), and ZO-1 (Stevenson et al., 1986; Barry et al., 1995; Joyce et al., 1998; Petroll et al., 1999). (2) gap junctional protein, which include connexin-43 (Jongen et al., 1991). These proteins are found between cells and located on the lateral plasma membranes anterior to the tight junctions (Iwamoto and Smelser, 1965; Leuenberger, 1973; Kreutziger, 1976; Raviola et al., 1980). These junctions are sites of dye and electrical coupling between neighboring cells (Rae et al., 1989). (3) adherens-type junctions which include N-(neuronal) cadherin (Beebe and Coats, 2000; Ickes et al., 2002), E-(epithelial) cadherin (Ickes et al., 2002), and α-, β-, and γ-catenin (plakoglobin) (Petroll et al., 1999). These adhesion junction-associated proteins mediate close contact between plasma membranes of adjacent cells and the underlying actin filament network, thereby strengthening cell–cell associations.

Although the anatomic structure of corneal endothelial junctions has been studied extensively, there are only limited studies on the regulation of these junctional molecules. The control of corneal endothelial junctions is clinically important for several reasons. First of all, it may control barrier functions and maintain stable corneal hydration status. In addition, it takes part in controlling the diffusion of nutrients from anterior chamber into avascular corneal stroma. Finally, it is involved in maintaining the non-proliferative status of confluent corneal endothelial cells due to the mechanism of contact inhibition. Because the adult human corneal endothelial cells are non-proliferative, marked decrease in corneal endothelial cellular counts due to aging, injury or diseases may lead to corneal endothelial decompensation and eventually corneal transplantation. Triggering adult corneal endothelial cells to reenter cell cycle by breaking through cell–cell contacts or other mechanism is thus clinically important because it may have the clinical applicability to avoid corneal transplantations.

Recently, it has been proposed that in certain endothelial and epithelial cells, the ability of cellular junctions to restrict paracellular flux is not immutable. Rather, a dynamic regulatory mechanism is found in controlling the gate function of cellular junctions (Madara, 1988; Rubin, 1992). Protein kinase C (Citi, 1993), cyclic AMP (Stelzner et al., 1989; Langeler and van Hinsbergh, 1991), extracellular calcium level (Martinez-Palomo et al., 1980; Gumbiner and Simons, 1986) and protein tyrosine phosphorylation (Matsuyoshi et al., 1992; Behrens et al., 1993; Young et al., 2003) were all proven to be involve in dynamic changes of tight junctions and adherens-type junctions. Among all, studies on extracellular calcium levels (Senoo and Joyce, 2000; Senoo et al., 2000) and protein tyrosine phosphorylation (Chen et al., 2005) have been applied to enhance corneal endothelial proliferation under the mechanism of disrupting cell–cell contact. Several adherens/gap/tight junction associated proteins that help maintain corneal endothelial cells in a non-reproliferative status are calcium sensitive. These calcium-dependent junctional proteins include the cadherins (adherens-type junction proteins) (Nagar et al., 1996; Pertz et al., 1999), occludin (tight-junction protein) (McCarthy et al., 1996), ZO-1 (tight junction-associated protein) (Siliciano and Goodenough, 1988), and connexin-43 (gap junction protein) (Jongen et al., 1991). Exposure of corneal endothelial cells to calcium-free medium was found to cause disruption of apical junctions, increase transendothelial perfusion and corneal edema (Kaye et al., 1968; Stern et al., 1981). Senoo et al. (2000) also demonstrated that treating corneal endothelial cells with ethylenediamine tetra-acetic acid (EDTA), a calcium chelator that may interfere with calcium sensitive junctional proteins, will release cell–cell contacts and trigger cell cycle entry in organ-cultured human donor corneas.

Protein tyrosine phosphorylation and dephosphorylation are important physiological mechanisms that control various cellular behaviors, such as proliferation, adhesion, and migration (Balsamo et al., 1998; Brady-Kalnay et al., 1993; Ostman et al., 1994; Pallen and Tong, 1991). Dephosphorylation events are mediated by a diverse group of enzymes, including protein tyrosine phosphatases (PTPs) (Barford et al., 1998). A number of studies suggest that PTPs are important in regulating the integrity of cell–cell contacts, including the assembly state of adherens-type junctions (Volberg et al., 1992; Müller et al., 1999; Zondag et al., 2000) and tight junctions. This role for PTPs is suggested by the finding that PTP activity is significantly higher in confluent than in subconfluent cells (Pallen and Tong, 1991; Gebbink et al., 1995). Volberg et al. showed that pervanadate, a general PTP inhibitor, elicited tyrosine phosphorylation of adherens-type junction in MDCK cells