Kaohsiung Journal of Medical Sciences (2014) 30, 1–11

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ORIGINAL ARTICLE

Pharmacological implications from the adhesion-induced signaling profiles in cultured human retinal pigment epithelial cells

Wen-Chuan Wu [a], Yo-Chen Chang [b], Kwou-Yeung Wu [a], Su-Yueh Chen [c], Ming-Chu Hsieh [a], Meng-Hsien Wu [a], Horng-Jiun Wu [a], Wen-Sheng Wu [d], Ying-Hsien Kao [e,*]

[a] *Department of Ophthalmology, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan*
[b] *Department of Ophthalmology, Kaohsiung Municipal Hsiao-Kang Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan*
[c] *Department of Nursing, Kaohsiung Medical University Chung-Ho Memorial Hospital, Kaohsiung, Taiwan*
[d] *Department of Laboratory Medicine and Biotechnology, College of Medicine, Tzu-Chi University, Hualien, Taiwan*
[e] *Department of Medical Research, E-Da Hospital, Kaohsiung, Taiwan*

Received 21 February 2013; accepted 6 May 2013
Available online 9 August 2013

KEYWORDS
Akt;
Extracellular matrix;
Fibronectin;
Integrin-linked kinase;
Mitogen-activated protein kinases

Abstract Extracellular matrix (ECM) plays an active and complex role in regulating cellular behaviors, including proliferation and adhesion. This study aimed at delineating the adhesion-induced signaling profiles in cultured human retinal pigment epithelium (RPE) cells and investigating the antiadhesion effect of antiproliferative drugs in this context. RPE R-50 cells grown on various ECM molecules, such as type I and IV collagens, fibronectin, and laminin, were used for adhesion assay and for examining the phosphorylation profiles of signaling mediators including Akt, extracellular signal-regulated kinase (ERK) 1/2, and integrin-linked kinase (ILK) using Western blotting. The cells receiving antiproliferative drug treatment at subtoxic doses were used to evaluate their antiadhesive and suppressive effects on kinase activities. ECM coating enhanced adhesion and spreading of RPE cells significantly. The cellular attachment onto ECM-coated surfaces differentially induced Akt, ERK1/2, and ILK phosphorylation, and concomitantly increased p53 phosphorylation and cyclin D1 expression, but

Conflicts of interest: The authors have no conflicts of interest relevant to this article.

* Corresponding author. Department of Medical Research, E-Da Hospital, No. 1 Yida Road, Yanchau District, Kaohsiung 824, Taiwan.
E-mail address: danyhkao@gmail.com (Y.-H. Kao).

http://dx.doi.org/10.1016/j.kjms.2013.06.002

decreased Bcl-2/Bax ratios. Treatment with antiproliferative agents, including 5-fluorouracil, mitomycin C, and daunomycin, at subtoxic doses suppressed the ability of RPE cells to adhere to ECM substratum significantly. This suppression was in part mediated through reduction of integrin β1 and β3 expressions and interfering Akt-ILK signaling activity. Mechanistically, blockade of PI3K/Akt signaling resulted in the suppressed adhesion of RPE cells to ECM. These findings support the hypothesis that, in addition to their antimitogenic effect, antiproliferative agents also exhibit suppressive effect on the adhesiveness of cultured RPE cells. Moreover, inhibitors of the PI3K/Akt signaling mediator can potentially be used as therapeutic agents for proliferative vitreoretinopathy.

Introduction

Proliferative vitreoretinopathy (PVR) is the most common cause for failure of retinal detachment surgery [1], and involves uncontrolled proliferation of non-neoplastic cells capable of forming cellular membranes on either side of the retina or along the detached surface of vitreous gel [2]. Etiologically, either previous retinal surgery, vitrectomy, preoperative PVR, large retinal tears with exposed retinal pigment epithelium (RPE), or preoperative choroidal detachment are the major causes of surgical failure [3]. Histological observations on surgically excised membranes reveal that RPE cells are one of the major cellular components involved in the pathogenesis of PVR [4,5]. RPE cells activated by proinflammatory cytokines at sites of injured tissues migrate into the vitreous cavity and generate fibrogenic mediators that remodel biosynthesis of the extracellular matrix (ECM) [6].

In addition to the cellular elements, PVR membranes consist of abundant ECM molecules, primarily collagen type I (CoI), collagen type IV (CoIV), fibronectin (FN), laminin (LM), and various glycosaminoglycans [7]. ECM plays an active role in regulating morphogenesis of cells, and multifunctionally influences their migration, proliferation, and metabolic function [8,9]. Contraction of PVR membranes creates a tractional force, which can distort or detach the retina [10]. Cell—ECM interaction has been demonstrated to be mediated by the cell surface adhesion receptor integrin. Integrin molecules are a group of highly homologous transmembrane proteins, consisting of a large extracellular domain, a hydrophobic membrane-spanning domain, and a smaller cytoplasmic domain. Each integrin contains one α and one β subunit. Upon ligation to ECM, the intracellular domain of integrin β subunit recruits an integrin-linked kinase (ILK) that conveys external stimuli into nuclei through activation of downstream kinase substrates, including Akt [11]. Tuning of the integrin—ILK axis signal cascade by phosphoinositide 3-kinase (PI3K) has been emphasized in the epithelial mesenchymal transdifferentiation of tumors [12] and RPE cells [13]. In fact, integrin immunoactivity has long been noted in PVR membranes [14]. Recent evidence reveals that integrin α5β1 mediates adhesion, migration, and proliferation of RPE cells [15], and that functional inhibition of integrin suppresses dedifferentiation of tumor cells [16] and adhesion of RPE cells to ECM substratum [17]. Similarly, targeting of ILK with a small interfering RNA suppresses PVR development through inhibition of attachment, spreading, migration, and proliferation of human RPE cells [13], highlighting the significance of integrin-related signaling in regulating RPE cell behaviors.

Seeding of human RPE cells onto ECM represents a suitable system to study the mechanisms underlying cell—ECM interactions. This study aimed at delineating the profiles of Akt, ERK1/2, and ILK phosphorylation in cultured human RPE cells seeded onto various ECM-coated surfaces. We also found that antiproliferative drugs, including 5-fluorouracil (5-FU), mitomycin C (MMC), and daunomycin (DM), prevented the adhesion of RPE cells via suppression of the critical signaling pathway.

Materials and methods

Reagents

Antiproliferative drugs including 5-FU, MMC, and DM were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ham's F12 nutrient mixture (F12 medium), fetal bovine serum, L-glutamine, trypsin—EDTA, and antibiotics were obtained from Invitrogen/Gibco BRL (Gaithersburg, MD, USA). The materials used for coating ECM were bovine serum albumin (BSA, Sigma-Aldrich), CoI (extracted from rat tail tendon as previously described) [18], CoIV (Southern Biotech, Birmingham, AL, USA), FN (Biomedical Technologies, Stoughton, MA, USA), and LM nonapeptide (Merck Calbiochem, San Diego, LA, USA). Kinase-selective inhibitors, including wortmannin for PI3K, SB203580 for p38 mitogen-activated protein kinase (p38 MAPK), PD98058 for MAPK/extracellular signal-regulated kinase (ERK) kinase 1 (MEK1), and SP600125 for c-Jun N-terminal kinase (JNK), were purchased from Sigma-Aldrich. All kinase inhibitors were stocked in Dimethyl sulfoxide (DMSO) at a concentration of 10 mM, and stored at −20°C. Antibodies against β-Actin, P53, phospho-P53 (Ser15), and integrin β1 and β3 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA), those against P21 and Bcl-2 from Millipore (Billerica, MA, USA), and antiproliferating cell nuclear antigen (anti-PCNA) from BD Biosciences (San Jose, CA, USA). Antibodies against Bax, cyclin D1, the phosphorylated form of Akt (Ser473), ILK (Ser246), and ERK1/2 (Thr185/Tyr187) were obtained from Cell Signaling Technology (Danvers, MA, USA).