Cancer Letters 391 (2017) 50–58

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Original Article

Prostaglandin E2 receptor 4 mediates renal cell carcinoma intravasation and metastasis

Yushan Zhang, Hamsa Thayele Purayil, Joseph B. Black, Francis Fetto, Lauren D. Lynch, Jude N. Masannat, Yehia Daaka*

Department of Anatomy and Cell Biology, University of Florida College of Medicine, Gainesville, FL 32610, USA

ARTICLE INFO

Article history:
Received 21 September 2016
Received in revised form
23 December 2016
Accepted 10 January 2017

Keywords:
EP4
CD24
Renal cell carcinoma
Intravasation
Metastasis

ABSTRACT

Treatment options for metastatic renal cell carcinoma (RCC) are limited. In this study, we investigated impact of prostaglandin E2 (PGE2) receptor 4 (EP4) on RCC metastasis. We found that knockdown of EP4 in two RCC cell lines, ACHN and SN12C, does not affect xenograft tumor take or growth rate in mice, but reduces metastasis by decreasing tumor intravasation. Using chick chorioallantoic membrane (CAM) assay, we confirmed that blockade of EP4 signaling inhibits tumor intravasation. In vitro studies associated EP4 expression and activity with RCC cell transendothelial migration (TEM). Gene expression analysis and validation assays showed that EP4 knockdown decreases expression of CD24, a ligand to the adhesion molecule P-selectin. Forced expression of CD24 in EP4 knockdown RCC rescues TEM capacity of the cells. Pharmacologic inhibition or knockdown of endothelial P-selectin blocks EP4-mediated cancer cell TEM, and inhibition of P-selectin prevents RCC tumor intravasation in CAM assay. Our results demonstrate that inhibition of EP4 attenuates the RCC intravasation and metastasis by downregulating CD24 and that P-selectin participates in tumor intravasation, implying a potential for these molecules as therapeutic targets for advanced RCC treatment.

Introduction

The incidence of kidney cancer is on the rise and risk factors include behavior (tobacco smoking), environment (exposure to radiation) and hereditary conditions (inactivation of von Hippel-Lindau gene). In 2016, an estimated 62,700 Americans will be diagnosed with kidney and renal pelvis cancer and 14,240 will die from the disease [1]. The most abundant type of kidney cancer is renal cell carcinoma (RCC) and more than 30% of RCC patients present with locally advanced and metastatic diseases at the time of diagnosis [1]. Moreover, nearly 30% of patients with localized disease will develop tumor recurrence and metastasis after surgical removal of the primary tumor mass [2].

Metastasis remains the major cause of mortality for RCC. Drugs targeting vascular endothelial growth factor (VEGF) and mammalian target of rapamycin (mTOR) pathways are the first-line treatment options for advanced RCC. Although initially effective, these targeted therapies usually fail within a year of administration, and patient response to these drugs is variable [3]. Hence elucidation of other signaling pathways and identification of alternative therapeutic targets are in demand to address this therapeutic gap.

In metastasis, cancer cells undergo multiple transitions, including loss of cell polarity and detachment from surrounding cells in the primary tumor mass, migration and invasion through basement membrane and stromal cell layers, intravasation into the vasculature, translocation and survival in the circulation, arrest at distant organs and extravasation into the parenchyma, and micrometastasis formation and colonization in target organs [4,5]. There are specific groups of molecules that function in concert in each step. For example, proteases are important for local invasion, whereas adhesion molecules like selectins and integrins are crucial for extravasation [6,7].

Proinflammatory cytokine prostaglandin E2 (PGE2) is a lipid product of cyclooxygenase 2 (COX2), and both COX2 and PGE2 are overexpressed in multiple cancers [8]. Experimental evidence

Abbreviations: CAM, chorioallantoic membrane; COX2, cyclooxygenase 2; EC, endothelial cell; EP4, E-type prostanoid receptor 4; IHC, immunohistochemistry; mTOR, mammalian target of rapamycin; PGE2, prostaglandin E2; RCC, renal cell carcinoma; SLN, sentinel lymph node; TEM, transendothelial migration; VASP, vasodilator-stimulated protein; VEGF, vascular endothelial growth factor.

* Corresponding author. 1333 Center Drive, B1-005, Gainesville, FL 32610, USA. Fax: +1 352 846 1248.
E-mail address: ydaaka@ufl.edu (Y. Daaka).

http://dx.doi.org/10.1016/j.canlet.2017.01.007
0304-3835/

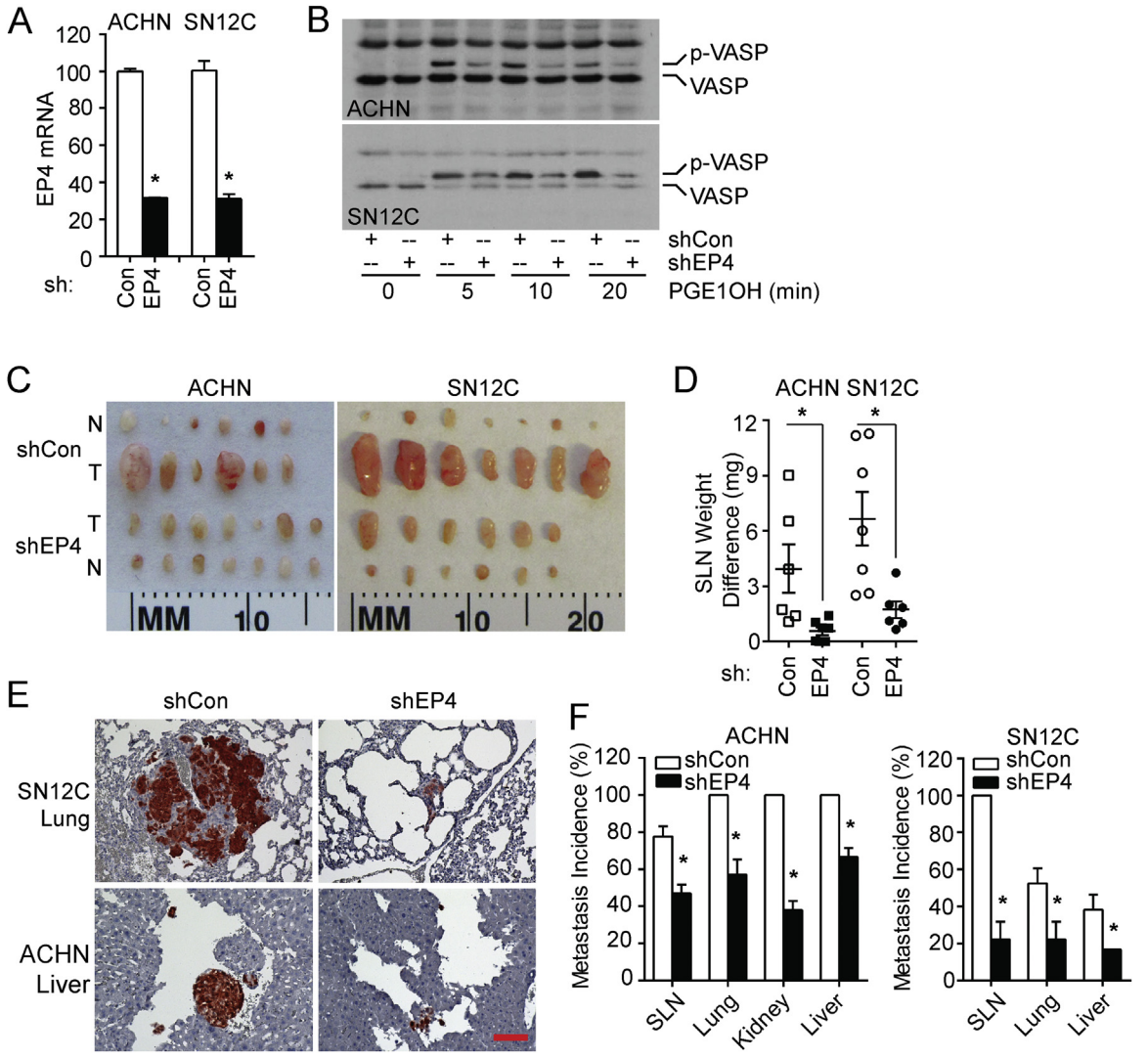

Fig. 1. Knockdown of EP4 expression inhibits ACHN and SN12C tumor metastasis. (A) ACHN and SN12C cells were transduced with shEP4 or control lentiviruses and screened to generate stable cell lines. EP4 mRNA expression was examined by quantitative PCR. (B) EP4 protein activity was verified by VASP phosphorylation, following stimulation with PGE1OH (1 μM for ACHN and 0.1 μM for SN12C). Experiments were repeated three times with similar results. (C) 1×10^6 stable EP4 knockdown (shEP4) ACHN (n = 7), SN12C (n = 6) or control (shCon) ACHN (n = 6), SN12C (n = 7) cells were implanted under the renal capsule of athymic nude mice and allowed to grow for 8 (ACHN) or 6 (SN12C) weeks. SLNs were isolated and weighed. N, contralateral renal lymph node. T, SLN. (D) SLN weight change was calculated by subtracting weight of the contralateral renal lymph node from the weight of SLN. (E) Representative IHC staining of lung with SN12C tumor and liver with ACHN tumor by anti-human LDHA. Bar: 100 μm. (F) ACHN and SN12C tumor metastasis incidence was calculated as the number of tumor cell positive organs divided by the number of total organs in that group. Shown is the average of three experiments. *In vivo* experiments were repeated twice with similar results. For all appropriate panels, *, $P < 0.05$, vs. indicated control.

establishes a role for PGE2 and its G protein-coupled receptor EP4 in cancer invasion and metastasis. For example, PGE2 stimulates colorectal cancer stem cell expansion and liver metastases by activating NF-κB through EP4-MAPK and EP4-PI3K signaling [9]. PGE2-activated EP4 enhances Akt and GSK3β phosphorylation and βcatenin-TCF4-LEF1 signaling, thereby promoting lung cancer invasion and metastasis [10]. EP4-Akt signaling also upregulates the expression of CCR7 and facilitates lymphatic invasion of breast cancer [11]. In MDA-MB-231 cells, activated EP4-EGFR-ERK1/2-Egr1 pathway increases Id1 transcription and the breast cancer cell invasion [12]. We recently showed that EP4-cAMP-Epac-Rap and EP4-Akt-RGC2-RalA signal transduction cascades promote the RCC cell invasion [13,14]. Little is known, however, about the role of EP4 in the exact steps of the metastatic cascade. The aim of this study was to reveal whether and how EP4 regulates RCC cell intravasation, thus providing rationale for targeting EP4 signaling pathway to treat metastatic RCC.

Materials and methods

Cell culture

Human renal cell carcinoma cell lines SN12C (National Cancer Institute) and ACHN (American Type Culture Collection) were maintained in RPMI 1640 medium (Corning) supplemented with 5% FBS (Thermo Scientific), 100 units/ml penicillin and 100 mg/ml streptomycin (Thermo Scientific). These cell lines were authenticated by STR-PCR (Genetica). HEK293T cells were maintained in DMEM medium (Corning) supplemented with 10% FBS. Neonatal human dermal microvascular endothelial cells (HMVECs; Lonza) were grown in endothelial growth medium-2 and were used within eight passages. Cells were cultured at 37 °C in a humidified 95% air, 5% CO_2 atmosphere.

Gene knockdown and overexpression

For stable knockdown of EP4 gene, HEK293T cells were transfected with pLKO.1 lentiviral vector TRCN0000000204 or TRCN0000000205 (Thermo Scientific) encoding shRNAs targeting human EP4. Lentiviral supernatants were collected, and used either separately or in combination to transduce ACHN and SN12C. Lentiviral vector targeting GFP was used to establish control cells. Cells were selected with 2 μg/ml puromycin (Sigma–Aldrich) commencing 48 h after transduction to