European Journal of Cancer (2013) 49, 3537–3546

Available at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.ejcancer.com

Clear cell renal cell carcinoma induces fibroblast-mediated production of stromal periostin

Nazihah Bakhtyar [a,b,c,d], Nicholas Wong [a,b,c,d], Anil Kapoor [b,c,d], Jean-Claude Cutz [c,e], Brianne Hill [a,b,c,d], Michelle Ghert [b], Damu Tang [a,b,c,d,*]

[a] Division of Nephrology, Department of Medicine, St. Joseph's Hospital, Hamilton, Ontario, Canada
[b] Department of Surgery, McMaster University, St. Joseph's Hospital, Hamilton, Ontario, Canada
[c] Father Sean O'Sullivan Research Institute, St. Joseph's Hospital, Hamilton, Ontario, Canada
[d] The Hamilton Center for Kidney Research, St. Joseph's Hospital, Hamilton, Ontario, Canada
[e] Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, Canada

Available online 26 July 2013

KEYWORDS
Periostin
Tumourigenesis
ccRCC

Abstract ***Objectives:*** Increase in periostin (PN) was reported in clear cell renal cell carcinoma (ccRCC). But how PN contributes to ccRCC pathogenesis remains unclear. This research will investigate the underlying mechanism.

Methods: The PN protein in 37 adjacent non-tumour kidney (ANK) tissues, their respective ccRCCs, 16 cases of metastasised ccRCC and xenograft tumours was analysed by immunohistochemistry. PN expression in ccRCC cells and NIH3T3 fibroblasts was examined by real time PCR (polymerase chain reaction) and western blot.

Results: PN was detected at low levels in the tubular epithelial cells of ANKs. PN was robustly increased in the ccRCC-associated stroma of both organ-confined and metastasised ccRCCs. Furthermore, despite A498 ccRCC cells and their-derived xenograft tumour cells expressing a low level of PN, a strong presence of stromal PN was observed especially in the boundary region between xenograft tumour mass and non-tumour tissue. Collectively, these results suggest that the ccRCC-associated PN was derived from stroma instead of tumours. This notion was supported by the co-existence of PN with α-smooth muscle actin (αSMA), a marker of activated fibroblasts, in both local and metastasised ccRCC. Furthermore, co-culture of NIH3T3 mouse fibroblasts with either human A498 or 786-0 ccRCC cells dramatically enhanced PN transcription only in NIH3T3 cells as well as NIH3T3 cell-mediated accumulation of extracellular PN. In return, extracellular PN significantly enhanced

* *Corresponding author:* Address: T3310, St. Joseph's Hospital, 50 Charlton Ave East, Hamilton, Ontario, Canada L8N 4A6. Tel.: +1 905 522 1155x35168; fax: +1 905 521 6181.
E-mail address: damut@mcmaster.ca (D. Tang).

http://dx.doi.org/10.1016/j.ejca.2013.06.032

A498 cell attachment. Elevation of PN promotes NIH3T3 cell proliferation and enhanced AKT activation.

Conclusions: ccRCC induces fibroblast-mediated accumulation of stromal PN; stromal PN enhances ccRCC cell attachment and fibroblast proliferation.

1. Introduction

Renal cell carcinoma (RCC) represents 3% and 4% of newly diagnosed malignancies in adult females and males, respectively.[1] The main histologic types of RCC consist of clear cell RCC (ccRCC, 75%), papillary RCC (pRCC, 12%), chromophobe RCC (cRCC, 4%) and oncocytoma (4%).[2] Among these types of RCC, ccRCC is the most aggressive form and causes most kidney cancer-associated deaths.[3]

Periostin (PN) is an extracellular matrix (ECM) protein, and contributes to RCC pathogenesis.[4,5] PN is a secreted cell adhesion protein with two functional regions. The N-terminal region (osteoblast-specific factor 2/OSF-2) consists of four repeats with homology to the insect axon guidance fasciclin I (FAS domain).[6] This region interacts with cellular integrins.[7] The C-terminal region of PN interacts with extracellular matrix proteins.[7–9] These structural features contribute to PN's role in the development of bone, teeth and heart.[10,11]

By binding to integrins, PN promotes cell motility, invasion and epithelial–mesenchymal transition during palatal fusion.[12] In line with these processes also being involved in tumourigenesis, PN enhances ovarian cancer motility.[13] PN was detected in a variety of human cancers.[14] More importantly, upregulation of PN was also observed in many human cancers, including cancer of the lung,[15,16] oesophagus,[17] colon,[18] liver,[19] pancreas,[20] thyroid,[21] ovary,[13] breast[22] and prostate.[23]

Consistent with the above observations, PN was detected in RCC at transcription and protein levels.[14,24] By using an elegant approach to identify kidney cancer antigens via ex vivo perfusion and biotinylation, PN was identified as the most abundant tumour-associated antigen.[4] In a most recent report, amplification of PN at the mRNA level was confirmed.[5] By examination of the PN protein using tissue microarray (TMA) containing 1007 RCC tumour tissues, PN expression was found to associate with aggressive features of RCC.[5] In line with these results, PN expression is associated with poor prognosis for RCC patients.[5] However, how PN promotes ccRCC tumourigenesis remains unclear.

To further investigate the role of PN in ccRCC pathogenesis, we analysed the PN protein in 37 local and 16 metastasised ccRCCs. Stromal PN was robustly upregulated in the organ-confined and metastasised ccRCC. Consistent with fibroblasts being the major type of stromal cells, ccRCC upregulated PN transcription in fibroblasts and enhanced the extracellular accumulation of PN. Elevated PN facilitates fibroblast proliferation and accumulated extracellular PN plays a role in ccRCC attachment.

2. Materials and methods

2.1. Collection of primary and metastatic ccRCCs, and cell lines

37 pairs of primary ccRCC and their respective non-tumour kidney tissues and 16 cases of metastasised ccRCC were collected in compliance with the local ethics regulations at St. Joseph's Healthcare Hamilton, Ontario, Canada.

The ccRCC cell lines 786-O and A498 were purchased from American Type Culture Collection and cultured in RPMI 1640 and MEM media supplemented with 10% Foetal Bovine Serum (FBS) and 1% Penicillin–Streptomycin (Invitrogen, Burlington ON).

2.2. Western blot analysis

Tissue and cell lysates were prepared according to our published conditions.[25] In brief, frozen renal cancer tissue and its corresponding ANK tissue were retrieved from liquid nitrogen and then crushed under liquid nitrogen with a mortar and pestil. Lysates were then prepared. 50 μg of total cell lysate was separated on sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) gel and transferred onto Immobilon-P membranes. Membranes were blocked with 5% skimmed milk and then incubated with the indicated antibodies at 4 °C overnight. Primary antibodies used were: anti-PN 1:1000 (Abcam) and anti-actin 1:1000 (Santa Cruz).

2.3. Immunohistochemistry (IHC) and dual immunofluorescence

IHC was performed on nine paraffin-embedded and serially-cut primary ccRCC and 16 metastatic ccRCC tissues as well as A498 cell-derived xenograft tumours according to our published procedure.[26,27] For dual immunofluorescence staining, anti-PN and anti-αSMA were used.

2.4. Retroviral infection

Retroviral infection was performed as we have previously described.[26,27] Briefly, a gag–pol expressing vector