MOLECULAR ONCOLOGY XXX (2015) 1–11

available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/molonc

An integrated genomic analysis of papillary renal cell carcinoma type 1 uncovers the role of focal adhesion and extracellular matrix pathways

Samantha Jane Wala[a,b], *Jason Raj Karamchandani*[b,1], *Rola Saleeb*[b], *Andrew Evans*[b,c], *Qiang Ding*[a], *Rania Ibrahim*[a], *Michael Jewett*[d], *Maria Pasic*[b,e], *Antonio Finelli*[d], *Kenneth Pace*[f], *Evi Lianidou*[g], *George Makram Yousef*[a,b,*]

[a]*The Keenan Research Centre in the Li Ka Shing Knowledge Institute, St. Michael's Hospital, 30 Bond Street, Toronto, Ontario M5B 1W8, Canada*

[b]*Department of Laboratory Medicine and Pathobiology, University of Toronto, 1 King's College Circle, Toronto, Ontario M5S 1A8, Canada*

[c]*Department of Pathology, Toronto General Hospital, Toronto, Ontario, Canada*

[d]*Department of Surgery, Princess Margaret Hospital, 610 University Avenue, Toronto, Ontario M5G 2M9, Canada*

[e]*Department of Laboratory Medicine, St. Joseph's Health Centre, 30 Queensway, Ontario M6R 1B5, Canada*

[f]*Department of Surgery, St. Michael's Hospital, 30 Bond Street, Toronto, Ontario M5B 1W8, Canada*

[g]*Laboratory of Analytical Chemistry, Department of Chemistry, University of Athens, 15771 Athens, Greece*

ARTICLE INFO

Article history:
Received 23 December 2014
Received in revised form
18 April 2015
Accepted 20 April 2015
Available online ■

Keywords:
Papillary renal cell carcinoma
Genetics
Focal adhesion
Extracellular matrix
miR-199a-3p
MET

ABSTRACT

Papillary renal cell carcinoma (pRCC) is the second most common RCC subtype and can be further classified as type 1 (pRCC1) or 2 (pRCC2). There is currently minimal understanding of pRCC1 pathogenesis, and treatment decisions are mostly empirical. The aim of this study was to identify biological pathways that are involved in pRCC1 pathogenesis using an integrated genomic approach. By microarray analysis, we identified a number of significantly dysregulated genes and microRNAs (miRNAs) that were unique to pRCC1. Integrated bioinformatics analyses showed enrichment of the focal adhesion and extracellular matrix (ECM) pathways. We experimentally validated that many members of these pathways are dysregulated in pRCC1. We identified and experimentally validated the downregulation of miR-199a-3p in pRCC1. Using cell line models, we showed that miR-199a-3p plays an important role in pRCC1 pathogenesis. Gain of function experiments showed that miR-199a-3p overexpression significantly decreased cell proliferation ($p = 0.013$). We also provide evidence that miR-199a-3p regulates the expression of genes linked to the focal adhesion and ECM pathways, such as caveolin 2 (*CAV2*), integrin beta 8 (*ITGB8*), *MET* proto-oncogene and mammalian target of rapamycin (*MTOR*). Using a luciferase reporter assay, we further provide evidence that miR-199a-3p overexpression decreases the expression of *MET* and

* *Corresponding author*. Department of Laboratory Medicine, St. Michael's, 30 Bond Street, Toronto, Ontario, M5B 1W8, Canada. Tel.: +1 416 864 6060x77605; fax: +1 416 864 5648.

E-mail addresses: Samantha.wala@gmail.com (S.J. Wala), jkaramch@gmail.com (J.R. Karamchandani), rola.saleeb@mail.utoronto.ca (R. Saleeb), andrew.evans@uhn.on.ca (A. Evans), DingQ@smh.ca (Q. Ding), IbrahimR@smh.ca (R. Ibrahim), m.jewett@utoronto.ca (M. Jewett), pasicm@stjoe.on.ca (M. Pasic), antonio.finelli@uhn.ca (A. Finelli), pacek@smh.ca (K. Pace), lianidou@chem.uoa.gr (E. Lianidou), yousefg@smh.ca (G.M. Yousef).

[1] Present address: Department of Pathology, McGill University Health Centre, 2155 Rue Guy, Montréal, Quebec H3G 1A4, Canada.

http://dx.doi.org/10.1016/j.molonc.2015.04.007

Please cite this article in press as: Wala, S.J., et al., An integrated genomic analysis of papillary renal cell carcinoma type 1 uncovers the role of focal adhesion and extracellular matrix pathways, Molecular Oncology (2015), http://dx.doi.org/10.1016/j.molonc.2015.04.007

MTOR. Using an integrated gene/miRNA approach, we provide evidence linking miRNAs to the focal adhesion and ECM pathways in pRCC1 pathogenesis. This novel information can contribute to the development of effective targeted therapies for pRCC1, for which there is none currently available in the clinic.

1. Introduction

Renal cell carcinoma (RCC)[2] constitutes the majority of renal neoplasms diagnosed in adults. This cancer is not a single disease, but instead encompasses a spectrum of subtypes (Delahunt et al., 2014; Ljungberg et al., 2010). Clear cell RCC (ccRCC) is the most common RCC subtype, whereas papillary RCC (pRCC) is second and can be further subdivided into type 1 (pRCC1) and 2 (pRCC2) (Delahunt et al., 2013; Delahunt and Eble, 1997; Ljungberg et al., 2010). pRCC1 is characterized by small cells with pale cytoplasm, whereas large cells and eosinophilic cytoplasm are hallmarks of type 2 (Delahunt and Eble, 1997). Only few molecular changes are reported for pRCC. Hereditary pRCC1 is associated with dysregulation in the *MET* proto-oncogene. In sporadic pRCC1, this gene can be mutated and over-expressed at the mRNA level (Lubensky et al., 1999; Sweeney et al., 2002). *MET* is an important transducer of downstream signaling pathways, including the focal adhesion and Akt signaling pathways (Chen and Chen, 2006; Xiao et al., 2001). In contrast, fumarate hydratase, a gene involved in the citric acid cycle, is commonly inactivated in hereditary pRCC2 (Toro et al., 2003). Gain of chromosome 17 is more frequently seen in type 1, and gain of 5q and loss of 1p and 3p are observed in type 2 (Klatte et al., 2009; Sanders et al., 2002). At the primary tumor level, pRCC2 has a worse prognosis than type 1 (Pignot et al., 2007). However, a study demonstrated that metastatic pRCC1 may be associated with a significantly worse survival outcome than metastatic pRCC2 (Klatte et al., 2009).

Due to the lack of understanding of the pathogenesis underlining pRCC, most patients are treated empirically using protocols designed for the clear cell subtype. There is currently no effective targeted therapy specific for patients with metastatic pRCC and no accurate prognostic models to predict its outcome. A thorough molecular understanding of the pathobiological mechanisms underpinning pRCC1 is a cornerstone towards that objective, which will lead into the new age of personalized medicine in kidney cancer (Pasic et al., 2013; White and Yousef, 2011).

A class of short, non-coding RNA molecules, called microRNAs (miRNAs), were first discovered by Victor Ambros and colleagues in 1993 (Lee et al., 1993). After two decades since their discovery, significant advances have been made in understanding the role of these endogenously expressed molecules in health and disease. miRNAs are highly conserved and are predominantly known for their ability to regulate expression of their target genes by base-pairing to the 3′ untranslated region (UTR) via the seed sequence. These regulatory molecules can downregulate mRNA expression by initiating mRNA decay or repress the initiation and post-initiation steps of translation (Mathonnet et al., 2007; Nottrott et al., 2006). Each miRNA is predicted to target hundreds of potential mRNAs (Hendrickson et al., 2009). Dysregulation of miRNA expression can abrogate a biological pathway and contribute to disease, including cancer. Recent evidence suggested the involvement of miRNAs in the clear cell subtype of RCC (Khella et al., 2013; Lichner et al., 2014; White et al., 2011) but little is known about their role in pRCC.

The focal adhesion and extracellular matrix (ECM) pathways are integral in maintaining cellular physiology. These pathways constitute a number of components that can mediate cell signal transduction to regulate processes, such as cell survival, proliferation and migration (Gilmore and Romer, 1996; Meredith, Jr. et al., 1993). These pathways have been shown to be dysregulated in a variety of malignancies, including breast cancer (Beaty et al., 2014), hepatocellular carcinoma (Ryu et al., 2014) and prostate cancer (Hensley et al., 2014). Emerging evidence suggests an important role for the focal adhesion and ECM pathways in RCC pathogenesis. For instance, RCC metastasis is facilitated by integrin-dependent adhesion to components of blood vessels via a chemokine receptor (Jones et al., 2007). Moreover, miRNAs have been reported to regulate the expression of members of the focal adhesion and ECM pathways (Sengupta et al., 2008). A recent report showed that miR-218 down-regulates the expression of caveolin 2 (*CAV2*), and transfection of this miRNA in A498 and 786-O cells led to significantly decreased cell proliferation, wound healing and cell invasion (Yamasaki et al., 2013).

The introduction of the concept of "integrated analysis" that combines multiple levels of molecular changes has revolutionized our understanding of RCC pathogenesis (Cancer Genome Atlas Research Network, 2013; Girgis et al., 2012). In the present study, using an integrated gene/miRNA approach, we provide evidence that links miRNAs to the focal adhesion and ECM pathways in pRCC1. We identified a number of uniquely dysregulated genes and miRNAs that are enriched in these biological pathways. We validated the differential regulation of members of these pathways on pRCC1 tissues. We also show that miR-199a-3p, which has decreased expression in pRCC1, targets members of the focal adhesion and ECM pathways, including *MET*. Finally, we provided evidence that miR-199a-3p may be an important tumor suppressor in pRCC1.

[2] **Non-standard abbreviations**: RCC; renal cell carcinoma, ccRCC; clear cell RCC, pRCC; papillary RCC, pRCC1; pRCC type 1, pRCC2; pRCC type 2, miRNAs; microRNAs, UTR; untranslated region, ECM; extracellular matrix, CAV2; caveolin 2, qRT-PCR; quantitative reverse transcription polymerase chain reaction, ITGB8; integrin beta 8, MTOR; mammalian target of rapamycin.

Please cite this article in press as: Wala, S.J., et al., An integrated genomic analysis of papillary renal cell carcinoma type 1 uncovers the role of focal adhesion and extracellular matrix pathways, Molecular Oncology (2015), http://dx.doi.org/10.1016/j.molonc.2015.04.007