Decreased apoptosis repressor with caspase recruitment domain confers resistance to sunitinib in renal cell carcinoma through alternate angiogenesis pathways

Glenda C. Gobe a, Keng Lim Ng a,e, David M. Small a, David A. Vesey a,b, David W. Johnson a,b, Hemamali Samaratunga a,c, Kimberley Oliver d, Simon Wood e, Johanna L. Barclay f, Retnagowri Rajandram a,g, Li Li h, Christudas Morais a,*

a Centre for Kidney Disease Research, School of Medicine, Translational Research Institute, The University of Queensland, Brisbane, Queensland, 4102, Australia
b Department of Renal Medicine, The University of Queensland at Princess Alexandra Hospital, Brisbane, Queensland, 4102, Australia
c Aquesta Pathology, Brisbane, Australia
d Anatomical Pathology, Princess Alexandra Hospital, Woolongabba, Queensland, Australia
e Department of Urology, Princess Alexandra Hospital, Woolongabba Queensland, Australia
f Mater Research Institute, University of Queensland, Australia
g Department of Surgery, Faculty of Medicine, University Malaya, Kuala Lumpur, Malaysia
h Laboratory of Translational Cancer Research, Ochsner Health System, New Orleans, LA, USA

Article info
Article history:
Received 3 March 2016
Accepted 10 March 2016
Available online 17 March 2016

Keywords:
Angiogenesis
ARC
Drug resistance
Renal cell carcinoma
Sunitinib

Abstract
Apoptosis repressor with caspase recruitment domain (ARC), an endogenous inhibitor of apoptosis, is upregulated in a number of human cancers, thereby conferring drug resistance and giving a rationale for the inhibition of ARC to overcome drug resistance. Our hypothesis was that ARC would be similarly upregulated and targetable for therapy in renal cell carcinoma (RCC). Expression of ARC was assessed in 85 human RCC samples and paired non-neoplastic kidney by qPCR and immunohistochemistry, as well as in four RCC cell lines by qPCR, Western immunoblot and confocal microscopy. Contrary to expectations, ARC was significantly decreased in the majority of clear cell RCC and in three (ACHN, Caki-1 and 786-0) of the four RCC cell lines compared with the HK-2 non-cancerous human proximal tubular epithelial cell line. Inhibition of ARC with shRNA in the RCC cell line (SN12K1) that had shown increased ARC expression conferred resistance to Sunitinib, and upregulated interleukin-6 (IL-6) and vascular endothelial growth factor (VEGF). We therefore propose that decreased ARC, particularly in clear cell RCC, confers resistance to targeted therapy through restoration of tyrosine kinase-independent alternate angiogenesis pathways. Although the results are contrary to expectations from other cancer studies, they were confirmed here with multiple analytical methods. We believe the highly heterogeneous nature of cancers like RCC predicate that expression patterns of molecules must be interpreted in relation to respective matched non-neoplastic regions. In the current study, this procedure indicated that ARC is decreased in RCC.

© 2016 Elsevier Inc. All rights reserved.

1. Introduction
Renal cell carcinoma (RCC) is a highly metastatic, heterogeneous, and treatment-resistant cancer with over 50 pathological entities [1,2]. The most common subtype is clear cell RCC (ccRCC, 70–80%), followed by papillary RCC (pRCC, 10–15%), chromophobe RCC (chRCC, 5%) and collecting duct RCC (<1%) [3]. Despite the introduction of many targeted therapies in clinical practice [4] metastatic RCC remains an incurable terminal disease. About 30% of patients do not respond to targeted therapy, and the remaining 70%, who initially respond, will develop resistance between 6 and 11 months [5,6].
Resistance to treatment, although multifactorial, is largely driven by defective apoptotic pathways. There are two well-known apoptotic pathways: extrinsic and intrinsic [7]. The extrinsic pathway is mostly initiated by death ligands, which form a death-inducing signalling complex (DISC). The intrinsic pathway is largely initiated by drugs, especially chemotherapeutics, which alter the mitochondrial membrane potential through the translocation of the cytoplasmic pro-apoptotic molecule Bax to the mitochondrial membrane transition pores [8]. Both extrinsic and intrinsic pathways converge at caspase-3, and its cleavage executes apoptosis. These processes are tightly-regulated by many endogenous apoptosis inhibitors, of which apoptosis repressor with caspase recruitment domain (ARC) is one. Most of the endogenous apoptosis inhibitors either inhibit the intrinsic or the extrinsic pathways, but ARC is unique in that it inhibits both pathways [8].

In 2008, Heikus and colleagues [9], using 47 RCC and adjacent non-neoplastic regions of the kidney, reported increased ARC mRNA in RCC when compared with adjacent non-neoplastic areas of the kidneys. In 2014, Razorenova et al. [10] also showed that ARC mRNA in RCC when compared with adjacent non-neoplastic areas of the kidney, reported increased ARC expression of 48 human RCC samples along with paired non-neoplastic regions. These results offered a rationale for further exploration of the molecular mechanisms of drug resistance in RCC secondary to ARC up-regulation.

The primary aim of the present investigation was to study the molecular mechanisms of ARC-mediated resistance of RCC to targeted therapies. As a first step, it was decided to investigate the expression of ARC in 48 human RCC samples along with paired non-neoplastic kidney samples by immunohistochemistry. Contrary to expectations, we found ARC protein was significantly decreased in the majority of RCC samples, particularly clear cell RCC, compared with paired normal kidney. This report therefore details our further analyses: ARC mRNA expression in a different paired set of 37 RCC samples; mRNA and protein in 4 RCC cell lines with and without Sunitinib therapy compared with the HK-2 human proximal tubular epithelial cell line; confocal microscopy to investigate protein localization in the cell lines; and inhibition of ARC with shRNA in an ARC-overexpressing cell line.

2. Materials and methods

2.1. Ethics approval

Approvals for the use of human tissue samples were obtained from the Human Research Ethics Committee of the Princess Alexandra Hospital (PAH) and the Human Ethics Committee of University of Queensland, Brisbane, Australia. Written informed consent was obtained from patients before the collection of samples (Ethics approval numbers 2006/189 and HREC/05/QPAH/95).

2.2. Tissue microarray and immunohistochemistry

Forty-eight formalin-fixed paraffin-embedded (FFPE) archival kidney cancer and matched morphologically normal regions of the kidneys collected from patients who underwent nephrectomy for kidney cancer between 1990 and 2011 at the PAH were used in this study. None of the patients received prior treatment before nephrectomy. Tumour grade and stage were determined by Fuhrman criteria and Tumour Node Metastasis (TNM) classification, respectively, by a qualified pathologist. Tissue cores (0.6 mm in diameter) were punched from selected areas of the paraffin blocks with core punch needles (Beecher Instruments, Inc. Sun Prairie, WI, USA) and tissue microarrays (TMA) were constructed using a Galileo TMA CK3000 Tissue Microarrayer (Fantioli, Milan, Italy) as previously described [11]. The TMAs were batch-immunostained for ARC with a validated antibody (abcam; ab118929) as previously described [11]. Digital images of the entire TMA were captured using the Aperio ScanScope XT Slide Scanner (Aperio Technologies, Vista, CA, USA) under 20× objective magnification. A quantitative scoring of ARC expression was analysed using the positive pixel algorithm of Aperio Imagescope [11].

2.3. ARC mRNA expression studies

Thirty-seven RCC and morphologically normal regions of matched kidneys were collected from patients who underwent nephrectomy for kidney cancer between June 2013 and December 2014 at the PAH. None of the patients received prior treatment before nephrectomy. The samples were snap-frozen in liquid nitrogen and stored at −80 °C until analysis. RNA was isolated using RNeasy Fibrous Tissue Mini Kit, following the instructions of the supplier (Qiagen, Maryland, USA). cDNA was synthesised using a High Capacity cDNA Reverse Transcription Kit (Life Technologies). Fully validated TaqMan Gene Expression Assay for ARC (Hs00608346_m1) was used with the SensiFAST™ Probe No-ROX Kit (Bioline, London, UK) in a LightCycler 480 (Roche Applied Science, Penzberg, Germany) to determine relative gene expression by the comparative Ct method. The TATA box binding protein (TBP Hs00427620_m1; Life Technologies) was used as internal control.

2.4. Cell culture

Metastatic human RCC cell lines ACHN, Caki-1 and 786-0, and the immortalised normal human proximal tubular epithelial cell line HK-2, were obtained from the American Type Culture Collection (ATCC), Rockville, MD, USA. Another human metastatic RCC cell line, SN12K1, was obtained from Professor D Nicol, formerly at PAH, Brisbane, Australia, through his collaborations with Dr IJ Fidler, Cancer Research Institute, MD Anderson Cancer Center, Orlando, FL, USA. The cell lines were cultured in DMEM/F12 supplemented with 10% fetal bovine serum, 50 U/ml penicillin and 50 μg/ml streptomycin (Gibco, Invitrogen, CA, USA) at 37 °C in an atmosphere of 95% air and 5% carbon dioxide.

2.5. qPCR and western blotting of cell lines

Cells were grown to approximately 90% confluence in 10 cm petri dishes. The culture medium was removed and the cells were washed in 1× ice-cold phosphate buffered saline (PBS). RNA was isolated using RNeasy Mini Kit, following the instructions of the supplier (Qiagen, Maryland, USA). The remaining qPCR methods were as for tissue, described previously. For protein, whole cell lysates were prepared by lysing the cells in 300 μl of radio immunoprecipitation assay (RIPA) buffer (Sigma–Aldrich, Missouri, USA), followed by centrifugation at 13,000 rpm for 15 min at 4 °C. The supernatants were collected and the protein contents measured using bichiorcinonic acid (BCA) protein assay reagent (Pierce, Rockford, IL, USA). The lysates were aliquoted and stored at −80 °C until further use. The proteins (50 μg) were resolved in 10% Tris–HCl gel and electro-transferred into polyvinylidene fluoride membranes (Millipore Corporation, MA, USA). Equal loading of proteins was confirmed by staining the membrane with Ponceau S solution. Standard Western blotting procedures were followed. The primary antibody was used at a dilution of 1:1000 and the secondary antibody at a dilution of 1:5000. The signals were detected by Super Signal West Pico Chemiluminescent Substrate (Pierce, Rockford, IL, USA). The differences in intensities of the signals were analysed by ImageJ software (National Institutes of Health, Bethesda, MD). The results are expressed as the ratio of house-
keeping protein GAPDH.

2.6. Cell viability

Cell viability was measured using 3-[4,5-dimethylthiazol-2-y]-2,5-diphenyltetrazolium bromide (MTT) (Sigma, Missouri, USA). Cells were grown in 96-well culture plates (2 × 10³ in 100 µl) overnight and treated with various concentrations of Sunitinib. Twenty four hours later, 5 µl of MTT solution (5 mg/ml in PBS) were added to each well and incubated at 37 °C for 90 min. The culture medium was removed and the purple crystals formed were dissolved in 100 µl of dimethyl sulfoxide (DMSO). The absorbance was measured in a Multiscan Go Microplate Reader (Thermo Scientific, Waltham, MA, USA) at 570 nm with a background correction at 690 nm.

2.7. Confocal microscopy

The cells (2 × 10³) were grown on glass cover slips overnight and washed in PBS, fixed in 1% paraformaldehyde for 15 min at room temperature, permeabilized in 0.1% Triton X-100 for 3 min, washed in TBS, and blocked with serum-free protein block (DakoCytomation, CA, USA) at room temperature for 1 h. The primary antibody was diluted (1:1000) in antibody diluent (DakoCytomation, CA, USA) at room temperature for 1 h. The primary antibody was labelled with FITC-conjugated secondary antibodies (Life Technologies) for 30 min, washed in PBS and mounted in Vectashield (Vector Laboratories Inc, CA, USA; Catalogue number, H-1200). The cells were observed under Zeiss 510 META confocal, using Zen 2010 software.

2.8. shRNA experiments

SureSilencing shRNA Plasmid Hygromycin kit for ARC was obtained from Qiagen (Maryland, USA). SN12K1 cells were transfected with Attractene Transfection Reagent (Qiagen) and an ARC-targeted from Qiagen (Maryland, USA). SN12K1 cells were transfected with Attractene Transfection Reagent (Qiagen) and an ARC-targeted from Qiagen (Maryland, USA). SN12K1 cells were transfected with Attractene Transfection Reagent (Qiagen) and an ARC-targeted from Qiagen (Maryland, USA). SN12K1 cells were transfected with Attractene Transfection Reagent (Qiagen) and an ARC-targeted from Qiagen (Maryland, USA). SN12K1 cells were transfected with Attractene Transfection Reagent (Qiagen) and an ARC-targeted from Qiagen (Maryland, USA). SN12K1 cells were transfected with Attractene Transfection Reagent (Qiagen) and an ARC-targeted from Qiagen (Maryland, USA). SN12K1 cells were transfected with Attractene Transfection Reagent (Qiagen) and an ARC-targeted from Qiagen (Maryland, USA). SN12K1 cells were transfected with Attractene Transfection Reagent (Qiagen) and an ARC-targeted from Qiagen (Maryland, USA). SN12K1 cells were transfected with Attractene Transfection Reagent (Qiagen) and an ARC-targeted from Qiagen (Maryland, USA). SN12K1 cells were transfected with Attractene Transfection Reagent (Qiagen) and an ARC-targeted from Qiagen (Maryland, USA). SN12K1 cells were transfected with Attractene Transfection Reagent (Qiagen) and an ARC-targeted from Qiagen (Maryland, USA). SN12K1 cells were transfected with Attractene Transfection Reagent (Qiagen) and an ARC-targeted from Qiagen (Maryland, USA). SN12K1 cells were transfected with Attractene Transfection Reagent (Qiagen) and an ARC-targeted from Qiagen (Maryland, USA). SN12K1 cells were transfected with Attractene Transfection Reagent (Qiagen) and an ARC-targeted from Qiagen (Maryland, USA). SN12K1 cells were transfected with Attractene Transfection Reagent (Qiagen) and an ARC-targeted from Qiagen (Maryland, USA).

2.9. Statistics

The data were analysed using student’s t-test and p < 0.05 was considered significant. The results are expressed as mean ± standard error of mean.

3. Results

3.1. ARC is decreased in ccRCC – TMA findings

Of the 48 matched-pair samples, 34 (70.83%) were ccRCC, 6 (12.5%) were chRCC, 7 (14.58%) were unclassified and 1 (2.08%) was collecting duct RCC. The patient characteristics are presented in Table 1. When compared with morphologically normal samples, the overall expression of ARC was significantly decreased in ccRCC (Fig. 1A). As can be expected of a highly heterogeneous cancer like RCC, dot blots showed that some samples had higher expression of ARC, while others lower expression, when compared with respective morphologically normal samples (Fig. 1B). When they were analysed separately, 23 samples (67.34%) had decreased expression (Fig. 1C) and the remaining 11 samples (32.35%) had increased expression of ARC (Fig. 1D). A representative image of a morphologically normal region and a ccRCC showing decreased expression of ARC is shown in Fig. 1E and F, respectively. Similarly, a representative image of a morphologically normal region and a ccRCC showing increased expression is shown in Fig. 1G and H, respectively. Images of liver cores, used as positive and negative controls, to test the specificity of antibody, are shown in Fig. 1I and J, respectively. The non-clear cell subtypes did not show any significant difference in expression when compared with their morphologically normal renal samples (not shown).

3.2. ARC is decreased in ccRCC – mRNA findings

To further verify these findings, mRNA expression of ARC was performed in a separate set of 37 snap-frozen RCC samples along with matched normal samples. The characteristics of these samples are presented in Table 2. Of the 37 samples, 22 (59.45%) were ccRCC, 4 (10.81%) were chromophobe and 3 (8.1%) were papillary. The remaining 8 samples were broadly classified as ‘benign other’ (BO; 4 samples; 10.81%; Table 2) and malignant other (MO, 4 samples; 10.81%; Table 2). In line with the TMA results, there was a significant decrease in the expression of ARC in ccRCC samples (Fig. 2A, overall expression; B, individual data). These samples also had a subpopulation (15/22; 68.18%) with decreased expression and a subpopulation (7/22; 31.82%) with increased expression. Remarkably, these values are similar to the TMA findings where 67.34% of RCC had a decreased expression and 32.35% of samples had an increased expression. Because of small sample number, the non-clear cells were combined together, and no statistically significant difference in ARC expression was observed (Fig. 2C), a finding similar to the TMA.
**Fig. 1. ARC is decreased in clear cell RCC (ccRCC).** (A) Overall expression of ARC is significantly decreased in ccRCC. (B) Dot blots showing individual raw data. When analysed separately based on expression patterns, 67.34% of ccRCC showed decreased expression (C), and 32.35% of ccRCC showed increased expression (D). Representative morphologically-normal region (E), and matched ccRCC (F) showing decrease in expression of ARC. Representative morphologically-normal region (G), and matched ccRCC (H) showing an increase in expression of ARC. I and J, liver core as positive and negative controls, respectively. **p < 0.01 and ***p < 0.001.

**Table 2**

Summary of clinical characteristics of snap frozen samples.

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td>Male = 19 (51.4%) Female = 18 (48.6%)</td>
</tr>
<tr>
<td>n = 37</td>
<td></td>
</tr>
<tr>
<td><strong>Age mean (range)</strong></td>
<td>55 (28 – 84)</td>
</tr>
<tr>
<td><strong>Laterality</strong></td>
<td>Right = 21 (56.8%) Left = 15 (40.5%)</td>
</tr>
<tr>
<td>Renal transplant = 1 (2.7%)</td>
<td></td>
</tr>
<tr>
<td><strong>Size mean, range (cm)</strong></td>
<td>5 (1.8 – 17)</td>
</tr>
<tr>
<td><strong>Clinical presentation</strong></td>
<td>Incidental = 22 (59.5%)</td>
</tr>
<tr>
<td>a Localised = 11 (29.7%)</td>
<td></td>
</tr>
<tr>
<td>b Constitutional = 1 (2.7%)</td>
<td></td>
</tr>
<tr>
<td>Unknown = 3 (8.1%)</td>
<td></td>
</tr>
<tr>
<td><strong>Nephrectomy type</strong></td>
<td>Partial = 4 (10.8%) Radical = 33 (89.2%)</td>
</tr>
<tr>
<td><strong>Stage (TNM) Tumour</strong></td>
<td>T1 = 23 (62.1%)</td>
</tr>
<tr>
<td>T2 = 9 (24.3%)</td>
<td></td>
</tr>
<tr>
<td>T3 = 4 (10.8%)</td>
<td></td>
</tr>
<tr>
<td>T4 = 1 (2.7%)</td>
<td></td>
</tr>
<tr>
<td><strong>Node</strong></td>
<td>N0 = 34 (91.9%)</td>
</tr>
<tr>
<td>N1 = 3 (8.1%)</td>
<td></td>
</tr>
<tr>
<td><strong>Metastases</strong></td>
<td>M0 = 37 (100%)</td>
</tr>
<tr>
<td><strong>Histopathology</strong></td>
<td>Clear cell RCC = 22 (59.5%) Papillary RCC = 3 (8.1%) Chromophobe RCC = 4 (10.8%)</td>
</tr>
<tr>
<td>a Malignant others = 4 (10.8%)</td>
<td></td>
</tr>
<tr>
<td>d Benign others = 4 (10.8%)</td>
<td></td>
</tr>
<tr>
<td><strong>Fuhrman grade (clear cell RCC and papillary RCC)</strong></td>
<td>Grade 1 = 4/25 (16%) Grade 2 = 6/25 (24%) Grade 3 = 11/25 (44%) Grade 4 = 4/25 (16%)</td>
</tr>
<tr>
<td>a None = 12</td>
<td></td>
</tr>
</tbody>
</table>

a Localised = haematuria, loin pain, loin mass.
b Constitutional = weight loss, loss of appetite, fever etc.
c Malignant others = 2 clear cell tubulopapillary, 1 urothelial carcinoma, 1 metastatic carcinosarcoma.
d Benign others = 2 benign cystic nephroma, 1 multiloculated cyst, 1 benign renomedullary interstitial cell tumour.
e Chromophobe, 4 malignant others and 4 benign others.
3.3. ARC is decreased in RCC cell lines

The expression of ARC in four human RCC cell lines was tested by qPCR. In comparison with non-cancerous human proximal tubular epithelial HK-2 cells, three of four cell lines (Caki-1, 786-0 and ACHN) showed a significant decrease in mRNA expression when compared with HK-2 (Fig. 3A). SN12K1 had the highest expression, exceeding that of HK-2 (Fig. 3A). All four cell lines were originally
derived from ccRCC. Thus the cell lines also reflect the disparate pattern that was observed in human samples. Furthermore we tested the protein expression of ARC in these cell lines by Western blotting, which also confirmed decreased expression of ARC in the three RCC cell lines. The quantification of the intensity as a ratio of ARC/GAPDH, and a representative blot, are given in Fig. 3B and C, respectively. Furthermore the in situ expression of ARC was studied by confocal microscopy. These findings also confirmed decreased expression of ARC in the majority of the RCC cells compared with HK-2 (Fig. 3D).

3.4. Decrease in ARC confers drug resistance and upregulates VEGF and IL-6

To study the biological significance of decreased ARC, we down-regulated ARC in SN12K1 cells using shRNA. One hygromycin-resistant stable clone showed a 26% reduction in ARC expression (Fig. 4A). These cells were treated with Sunitinib, the first-line targeted therapy for metastatic RCC. When compared with scrambled shRNA, ARC down-regulated cells demonstrated a 2.4-fold increase in IC50 value (2.7 nM Sunitinib for scrambled ShRNA cells vs. 6.4 nM for ARC down-regulated cells). When the experiments were repeated eight weeks later after multiple passages of the cells, the difference in RNA was 21%, and the IC50 value for scrambled shRNA was 3.5 nM, and for ARC down-regulated cells was 8.6 nM (data not shown). Thus, a 25% decrease in ARC was associated with approximately 2-fold increase in drug resistance. To explore the possible mechanism behind the drug resistance, we studied the expression of VEGF-A, IL-6 and IL-8 by qPCR. A 0.25 fold increase VEGF (Fig. 4B) and a 3-fold increase in IL-6 (Fig. 4C) were observed. No marked changes in IL-8 were observed (not shown).

4. Discussion

The results of this study show that ARC is significantly decreased in the majority of ccRCC in our cohorts. Decreased ARC also conferred resistance to Sunitinib in vitro. These findings are contradictory to what was anticipated based on the available literature [9,10] and highlight the importance of using paired normal kidney samples as controls to account for heterogeneity. For a highly heterogeneous disease like RCC with ever expanding subtypes [2], the expression pattern of any molecule must be interpreted in terms of its heterogeneity. Heikaus and colleagues [9] studied ARC mRNA expression in 47 ccRCC and matched non neoplastic samples by qPCR. They also performed Western blotting of 9 arbitrarily selected RCC and 3 non-neoplastic regions. Both assays showed an increase in expression of ARC in the RCC. While the increased expression by Western blotting could be attributed to the lack of matched pairs, the reason for increased expression by qPCR is not clear, however, there is one possible scenario. The RCC samples were pooled together according to their grades and compared with the expression of all normal samples. As such, the predominant RCC samples may have belonged to an ‘ARC high’ group. It is not clear if a comparison of RCC samples with matched normal samples may have yielded a different outcome, for example two different populations, with increased and decreased expression patterns, as observed in our study. Whatever the reason, these discrepancies highlight the importance of interpreting molecular expression pattern in human samples in the context of heterogeneity of RCC. In addition, we believe that the expression pattern of molecules should be interpreted in relation to matched non-neoplastic regions.

The report by Razorenova and colleagues [10] used two public
domain gene expression microarray data sets and compared just 10 normal (non-matched) samples with 177 tumour tissues. Furthermore, they used commercially available kidney tissue arrays, which according to Fig. 4F in that report, had 9 normal samples and 57 tumour samples. Of these samples, 37 had a higher expression of ARC by immunohistochemistry. This means 20 samples, more than twice the number of normal samples, had lower expression. Thus, it is possible that lack of an adequate number of matched normal samples led to their conclusion that ARC is increased in RCC.

To study the clinical relevance of decreased ARC expression in RCC, ARC was down-regulated by the shRNA technique. The clone that was developed had a 26% reduction of ARC. This 26% decrease in ARC was sufficient to confer more than two-fold resistance to Sunitinib therapy. This could, at least in part, be a reason why 30% of RCC are inherently resistant to targeted therapy. The mechanisms of Sunitinib resistance are varied and multifactorial [12]. Based on the emerging knowledge, activation of tyrosine kinase-independent alternate angiogenesis pathways leading to restoration of angiogenesis appears to be gaining consensus [12–14]. To explore the potential molecular mechanisms of Sunitinib resistance with decreased ARC, we studied the expression of VEGF, IL-6 and IL-8, which have been implicated in the development of resistance to Sunitinib [15,16]. While there was no significant change in IL-8, there was a 3-fold increase in IL-6 in ARC-downregulated cells. IL-6 is a potent pro-inflammatory molecule that regulates VEGF [17]. Thus a decrease in ARC may lead to drug resistance via the activation of IL-6, which in turn restores angiogenesis through the activation of VEGF, as shown in Fig. 4D.

In conclusion, in the samples we have studied, always using matched normal kidney samples as a comparison, we find that ARC is decreased in the majority of ccRCC. For a highly heterogeneous cancer like RCC, the expression pattern of molecules should be interpreted in relation to matched non-neoplastic regions. If this is ignored, it is possible to reach a conclusion that ARC is increased in RCC. While the significance of increased ARC in some RCC samples is unclear, the decrease in ARC in the majority of RCC samples may have a clinical significance. Since decreased ARC conferred resistance to Sunitinib in vitro, it is possible to develop a personalised medicine approach in the future. Based on the ARC levels, it is possible to determine whether a patient will benefit from Sunitinib or other forms of tyrosine kinase inhibitors as first-line therapeutics. Further experiments are warranted to explore this aspect, and also to study the molecular mechanisms of ARC-mediated RCC progression and drug resistance.

Conflict of interest

Professor David W Johnson is the recipient of a Queensland Government Health Research Fellowship. He has received consultancy fees, research funds, speaking honoraria and travel sponsorships from Janssen-Cilag, Amgen, Pfizer and Roche. All other authors declare no other potential conflicts of interest with respect to research, authorship and/or publication of this article.

References