Expression patterns of RelA and c-mip are associated with different glomerular diseases following anti-VEGF therapy

Hassan Izzedine[1,8], Melanie Mangier[2,3,8], Virginie Ory[2,3], Shao-Yu Zhang[2,3], Kelhia Sendeyo[2,3], Khedidja Bouachi[2,3,4], Vincent Audard[2,3,4], Christine Péchoux[5], Jean C. Soria[6], Christophe Massard[6], Rastilav Bahleda[6], Edward Bourry[1], David Khayat[7], Alain Baumelou[1], Philippe Lang[2,3,4], Mario Ollero[2,3], Andre Pawlak[2,3] and Djillali Sahali[2,3,4]

[1]Department of Nephrology, Pitie-Salpetriere Hospital, Paris, France; [2]INSERM U 955, Equipe 21, Créteil, France; [3]Université Paris-Est Creteil, Créteil, France; [4]Service de Néphrologie, AP-HP, Groupe Hospitalier Henri Mondor-Albert Chenevier, Créteil, France; [5]INRA, UR1196 Génomique et Physiologie de la Lactation, Plateforme MIMA2, Jouy-en-Josas, France; [6]Institut Gustave Roussy, Villejuif, France and [7]Department of Medical Oncology, Pitie-Salpetriere Hospital, Paris, France

Renal toxicity constitutes a dose-limiting side effect of anticancer therapies targeting vascular endothelial growth factor (VEGF). In order to study this further, we followed up 29 patients receiving this treatment, who experienced proteinuria, hypertension, and/or renal insufficiency. Eight developed minimal change nephropathy/focal segmental glomerulopathy (MCN/FSG)–like lesions and 13 developed thrombotic microangiopathy (TMA). Patients receiving receptor tyrosine kinase inhibitors (RTKIs) mainly developed MCN/FSG-like lesions, whereas TMA complicated anti-VEGF therapy. There were no mutations in factor H, factor I, or membrane cofactor protein of the complement alternative pathway, while plasma ADAMTS13 activity persisted and anti-ADAMTS13 antibodies were undetectable in patients with TMA. Glomerular VEGF expression was undetectable in TMA and decreased in MCN/FSG. Glomeruli from patients with TMA displayed a high abundance of RelA in endothelial cells and in the podocyte nuclei, but c-mip was not detected. Conversely, MCN/FSG-like lesions exhibited a high abundance of c-mip, whereas RelA was scarcely detected. RelA binds *in vivo* to the *c-mip* promoter and prevents its transcriptional activation, whereas *RelA* knockdown releases *c-mip* activation. The RTKI sorafenib inhibited RelA activity, which then promoted c-mip expression. Thus, our results suggest that c-mip and RelA define two distinct types of renal damage associated with VEGF-targeted therapies.

Correspondence: *Hassane Izzedine, Service de Néphrologie, APHP, Université Pierre and Marie Curie, Paris VI, Groupe Hospitalier Pitié-Salpêtrière, Paris 75013, France. E-mail: hassan.izzedine@psl.aphp.fr or Djillali Sahali, Service de Néphrologie, APHP, Université Paris-Est Créteil, UMRS 955, Equipe 21, Groupe Hospitalier Henri Mondor-Albert Chenevier, Créteil, F-94010 France. E-mail: dil.sahali@inserm.fr*

[8]These authors contributed equally to this work.

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In renal glomeruli, podocytes express vascular endothelial growth factor (VEGF), whereas VEGF receptor tyrosine kinases (RTKs) are expressed by both podocytes and glomerular endothelial cells.[1] The biological functions of VEGF are mediated by its binding to one of the VEGF receptors (VEGFRs), which include VEGFR1 (Flt-1), VEGFR2 (KDR/Flk-1), and VEGFR-3 (Flt-4). The VEGF family comprises seven members: VEGF-A, -B, -C, -D, -E, and placenta growth factor 1 and 2. VEGF-A (also referred to as VEGF) binds to VEGFR1 and -2, whereas VEGF-C and -D bind to VEGFR2 and VEGFR-3, respectively. VEGFR2 expression has been reported in cultured podocytes.[2] Structurally, RTKs consist of an extracellular ligand–binding domain, a transmembrane region, and an intracellular kinase domain that mediates downstream signal transduction. Upon binding to their ligand, RTKs dimerize and are phosphorylated on their kinase domain, leading to the recruitment of adaptor proteins that trigger intracellular signaling cascades important for processes such as cell proliferation and survival, migration, and metabolism.[3] Dysregulation of RTK signaling by mutation or by ectopic receptor or ligand overproduction has been implicated in several aspects of tumor progression, including cell proliferation, survival, angiogenesis, and tumor dissemination.[4]

VEGFR2 is the predominant receptor in angiogenic signaling.[5,6] VEGF is upregulated in response to hypoxia, oncogenes, or cytokines, and its expression is associated with poor prognosis in several types of cancer.[7,8]

Experimental, preclinical, and clinical studies have identified angiogenesis as a key process in the progression of most solid tumors. Thus, inhibition of VEGF and platelet-derived

Table 1 | Patient characteristics at baseline

	All patients	MCN/FSG	TMA
n	29	8	13
Men	19	6	3
Age, years, mean (range)	55.2 (20–79)	71.5 (37–79)	69.5 (20–67)
mRCC	17	7	3
Previous nephrectomy	14	6	3
Previous radiotherapy and/or IFNα use	12	5	4
Anti-VEGF agents			
Bevacizumab (cumulative dose)	9	–	6 (10–240 mg/kg)
VEGF Trap (cumulative dose)	6	–	5 (12–54 mg/kg)
Sunitinib (cumulative dose)	11	5 (50–200 mg)	2 (100 mg)
Axitinib	1	1 (40 mg)	–
Sorafenib (cumulative dose)	3	2 (800 mg)	–
Renal parameters			
SBP, mm Hg, mean (range)	150.0 (110–190)	130.0 (110–180)	165.0 (120–190)
DBP, mm Hg, mean (range)	95.0 (60–115)	83.0 (60–110)	100.0 (80–115)
Proteinuria, g/day, mean (range)	3.50 (0.6–19.5)	3.5 (2–5.5)	4.11 (0.6–19.5)
Edema	14	5 (62.5%)	4(31%)
Microhematuria	12	2 (28.5%)	6 (46%)
SCr, mg/dl, mean (range)	1.26 (0.70–4.44)	0.95 (0.79–1.27)	0.86 (0.70–1.45)
aMDRD CrCl, ml/min per 1.73 m^2, mean (range)	76.5 (13.7–120)	80 (17–102)	75 (45–120)
Outcome			
Follow-up duration	1 mo–3 years	3 years	6 mo–2 years
Alive	14	1	8
SCr, mg/dl	1.0 (0.75–2.1)	1.20	0.95 (0.75–2.1)

Abbreviations: aMDRD CrCl, creatinine clearance; DBP, diastolic blood pressure; IFNα, interferon-α; MCN/FSG, minimal change nephropathy/focal segmental glomerulopathy; mo, month; mRCC, metastatic renal cell carcinoma; SBP, systolic blood pressure; SCr, serum creatinine; TMA, thrombotic microangiopathy; VEGF, vascular endothelial growth factor.

growth factor signaling is predicted to lead to anti-angiogenic effects and prevent the progression of tumors.[9,10] Therapeutic approaches targeting the VEGF ligand or RTK inhibitors (RTKIs) have recently been developed. Several antagonists of VEGF signaling are being tested in clinical trials, including bevacizumab (anti-VEGF monoclonal antibody) and RTKIs such as sunitinib, imatinib, and sorafenib.[11] Although RTKIs are widely used as inhibitors of VEGFRs, they interfere with the activity of other growth factors, such as platelet-derived growth factor receptors, stem cell factor receptor (c-kit), FMS-like tyrosine kinase-3 (Flt-3), b-raf, and Bcl-Abl. Thus, they are commonly named as multitargeted RTKIs and are widely used in medical oncology practice. However, renal complications constitute a dose-limiting side effect of RTKI and anti-VEGF therapies.

We report here on a series of 29 patients treated with anti-VEGF and RTKIs, who experienced proteinuria, hypertension, and/or renal insufficiency. Immunomorphological and molecular studies suggest that RelA and c-mip define two separate glomerular damages associated with anti-angiogenic drugs, based on two distinct pathophysiological mechanisms.

RESULTS

Clinical characteristics of patients with renal diseases

Baseline patient characteristics are summarized in Table 1. All patients were referred to a nephrology department because of discovery of proteinuria and/or increased serum creatinine following anti-VEGF initiating treatment. Sixteen patients had renal cell carcinoma and received 50 mg of sunitinib daily ($n = 11$), 400 mg of sorafenib ($n = 3$) or 5 mg axitinib ($n = 1$) twice daily, or bevacizumab (10 mg/kg/dose) twice monthly ($n = 2$). Thirteen other patients received VEGF Trap (4–6 mg/kg/dose) every 3 weeks (for ovarian, adrenal, breast, prostate, esophageal, and rectal cancers) or bevacizumab 10 mg/kg/dose twice monthly (for lung, uterine, and colorectal cancers). At the time of kidney biopsy, 20 patients (68,9%) presented with hypertension requiring antihypertensive treatment, and 14 (48,3%) with renal failure defined by a creatinine clearance rate below 60 ml/min per 1.73 m^2. Kidney biopsy was performed 2–12 weeks after the start of anti-VEGF treatment. The average number of glomeruli was 18.2 (range: 9–50). The principal pathological findings were thrombotic microangiopathy (TMA; $n = 13$), minimal change nephropathy/focal segmental glomerulopathy (MCN/FSG)–like syndromes ($n = 8$), acute tubular necrosis ($n = 3$), and one case of each of the following lesions: membranous nephropathy, IgA nephropathy, anti-neutrophil cytoplasmic antibody-negative pauci-immune crescentic glomerulonephritis, diabetic nephropathy, and acute interstitial nephritis. Fourteen patients (56%) died during the study because of cancer progression. Owing to the expected limited survival, managing oncologists were reluctant to repeat the urinary investigations.

MCN/FSG-like damages

Eight patients with a previous nephrectomy for metastatic renal cell carcinoma and/or interferon-α therapy exhibited