Translational Oncology
www.transonc.com

Volume 7 Number 2 April 2014 pp. 188–195 188

The Role of Angiopoietins as Potential Therapeutic Targets in Renal Cell Carcinoma[1]

Xiaoen Wang*,†, Andrea J. Bullock†, Liang Zhang†, Lin Wei†, Dongyin Yu‡, Kedar Mahagaokar†, David C. Alsop*, James W. Mier†, Michael B. Atkins§, Angela Coxon‡, Jon Oliner‡ and Rupal S. Bhatt†

*Department of Radiology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA; †Division of Hematology-Oncology and Cancer Biology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA; ‡Oncology Research, Amgen Inc, Thousand Oaks, CA; §Departments of Oncology and Medicine, Georgetown-Lombardi Comprehensive Cancer Center, Washington, DC

Abstract

Angiopoietin 2 (Ang2) is a secreted glycoprotein upregulated at sites of angiogenesis and has been implicated in cancer neovascularization. Recent studies have suggested efficacy of combined Ang and vascular endothelial growth factor receptor (VEGFR) inhibition for patients with metastatic renal cell carcinoma (mRCC). We measured Ang2 expression in human tissue and plasma, and tested the effect of dual Ang1/2 (trebananib; AMG386) or Ang2 alone (L1-7) inhibition with VEGFR inhibition on murine RCC growth and blood flow. Ang2 levels were higher in human tumors than normal tissues with RCC ranking highest for Ang2 expression across all tumor types tested. Plasma Ang2 was significantly higher in patients with mRCC compared to controls or patients with stage I disease. Plasma Ang2 decreased with sunitinib treatment and increased at time of disease progression. In the RCC mouse, dual Ang1/2 and Ang2 inhibition improved the activity of sunitinib. Combined Ang1/2 and VEGFR inhibition prevented the resumption of blood flow associated with sunitinib resistance. Thus, Ang2 inhibition, independent of Ang1 inhibition, improves the activity of sunitinib and plasma Ang2 increases in the setting of progression on sunitinib possibly contributing to resistance. Further, arterial spin-labeled perfusion magnetic resonance imaging might be a non-invasive marker of the antiangiogenic activity of Ang inhibitors.

Translational Oncology (2014) 7, 188–195

Introduction

Vascular endothelial growth factor receptor (VEGFR) inhibition has shown significant antitumor and antiangiogenic activity in patients with renal cell carcinoma (RCC). Agents such as sunitinib, sorafenib, pazopanib, and axitinib have all shown activities in patients with metastatic RCC [1-4] leading to Food and Drug Administration approval. However, antiangiogenic therapy with VEGFR tyrosine kinase inhibitors (TKIs) does not lead to durable or complete responses and treatment resistance develops at a median of 9 to 12 months.

Resistance could be associated with selection of tumor cells that can survive treatment-induced hypoxia or through activation of angiogenic pathways parallel to the VEGF axis. We have shown that resistance to therapy is associated with resumption of angiogenesis despite continued therapy, consistent with the activation of alternate angiogenic pathways [5,6]. Others have implicated angiogenic factors, such as interleukin 8 and fibroblast growth factor in resistance [7,8]. One additional pathway that has recently been the

Address all correspondence to: Rupal S. Bhatt, MD/PhD, Division of Hematology-Oncology and Cancer Biology, Beth Israel Deaconess Medical Center, 330 Brookline Avenue, Boston, MA 02215.

[1] This work was supported by the Dana-Farber/Harvard Cancer Center Kidney Cancer SPORE (Specialized Programs of Research Excellence) (NIH, National Institutes of Health, 2P50CA101942-08), NIH K08 CA138900-03 to R.S.B., and a grant from Amgen.
Received 19 September 2013; Revised 10 December 2013; Accepted 2 January 2014

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1936-5233/14 http://dx.doi.org/10.1016/j.tranon.2014.02.003

Table 1. The List of Primers Used in the Gene Expression Studies by RT-PCR.

	Forward Primer	Reverse Primer	Probe
Ang1	GGA GGA TGG TGG TTT GAT GC	TGG TTT TGT CCC GCA GTA TAG A	<6FAM>TG TGG CCC CTC CAA TCT AAA TGG AAT G<TAM>
Ang2	TAC ACT TTC CTC CTG CCA GAG AT	TGC ACA GCA TTG GAC ACG T	<6FAM>CA ACT GCC GCT CTT CCT CCA GCC <TAM>
CD31	CGG TGC AAA ATG GGA AGA A	TGA CGT GAG AGG TGG TGC TG	<6FAM>TG ACC CTG CAG TGC TTC GCG G<TAM>
VEGFR2	GAC AGA GGG ACT TGG ACT GGC	CTC AGT CAC CTC CAC CCT TTG	<6FAM>TG GCC CAA TAA TCA GAG TGG CAG TGA <TAM>
VEGF	GGG CAG AAT CAT CAC GAA GTG	GGT CTC GAT TGG ATG GCA GT	<6FAM>TG AAG TTC ATG GAT GTC TAT CAG CGC AGC <TAM>

subject of much investigation is the angiopoietin (Ang) axis. Ang2 inhibition has been shown to have activity in preclinical models and several agents are currently being tested in clinical settings across multiple tumor types [9-12]. While much is known about the role of Ang2 in cancer, several important unanswered questions exist. Ang1 and Ang2 are endothelial-secreted proteins with a complex relationship and potentially competing overall effects on tumor angiogenesis. Ang2 is most commonly described as a molecule that destabilizes vascular networks, supporting neoangiogenesis [13,14]. Ang1 binds to the Tie2 receptor to promote vascular maturation, inhibiting angiogenesis. Ang2 is an antagonist of Ang1 signaling through Tie2. Thus, one of the key questions in the Ang field is whether, in RCC, Ang1 inhibition undermines or augments effects of Ang2 inhibition.

In previous studies, the Ang2-specific inhibitor L1-7, Ang2-CovX bodies, and the Ang2 antibody 3.19.3 slowed the growth of colon and lung cancer xenografts and accentuated the activity of VEGF pathway inhibitors [10,15,16]. The dual Ang1/2 inhibitor, trebananib (AMG386), was found to have more activity than Ang2-specific inhibitors alone in colon cancer models [9]. Falcón et al. described similar findings in a colon cancer model and showed that Ang1 inhibition augmented the effect of Ang2 inhibition by preventing vascular normalization seen with the Ang2 inhibitor [13]. RCC is typified by Von Hippel–Lindau (VHL) loss leading to exquisite dependency on the VEGF-driven angiogenesis. As a consequence, RCC exposure to VEGF pathway inhibitors has been shown to result in "vascular infarction" rather than vascular normalization. Given this distinct biology, we sought to determine the relative effects on tumor growth and perfusion of Ang1, Ang2, and dual Ang1/2 inhibition alone and in combination with VEGF pathway inhibitors in a mouse model of RCC.

Another key question related directly to the clinical development of Ang inhibitors is how to select the patients most likely to benefit from this treatment. Currently, there is little data to guide optimal patient selection and determine the optimal treatment setting. To explore the possibility that Ang2 may be a useful surrogate or predictive marker of activity in RCC, we measured Ang2 plasma levels in patients with RCC either at presentation or during the course of VEGFR-targeted therapy. Taken together, these data inform the continued exploration of Ang2 inhibitors such as trebananib in patients with RCC or other cancers.

Materials and Methods

Evaluation of Gene Expression by Reverse Transcription–Polymerase Chain Reaction

Frozen tumor specimens of several human tumor types and non-malignant renal tissues, including non-malignant kidney tissue (cortex and medulla from non-oncology patients), clear cell RCC (ccRCC) tissue, and other non-renal tumor tissue including bladder, lymphoma, lung (adeno), lung (squamous), laryngeal, ovarian, prostate, gastric, breast, colorectal, and pancreatic tumors, were obtained. Total RNA was obtained either directly from a vendor (Ardais Corporation, Lexington, MA) or extracted from frozen tissue samples (Zoion Diagnostics Inc, Shrewsbury, MA) with the Qiagen RNeasy Mini Kit. All the RNA samples were treated with RNase-free DNase to remove genomic DNA before reverse transcription–polymerase chain reaction (RT-PCR). Quantitative RT-PCR was performed with 100 ng of total RNA in duplicate with a TaqMan EZ RT-PCR Kit from Roche (Indianapolis, IN). The primers and probes used in this study are listed in Table 1. *In vitro* transcripts of cDNA fragments for each gene were used as standard for calculating mRNA copy numbers. Cyclophilin A mRNA copy number was used for normalization.

Plasma Ang2 Measurement

Circulating Ang2 levels were measured in plasma collected from 50 patients with metastatic RCC, 39 patients with stage I RCC before nephrectomy, and 26 healthy volunteers. All 89 patients with RCC had histologically confirmed RCC (99% ccRCC, n = 88), and all provided written informed consent for sample collection. Samples were collected from healthy volunteers not being seen in any specialty clinics and who had no RCC pathology or urologic issues. Approval for the RCC sample collection protocol was obtained from the Institutional Review Board of the Dana-Farber/Harvard Cancer

Table 2. Characteristics of Patients Included in the Plasma Studies.

Patient Characteristics	Sunitinib (N = 44)	
Age	57 (Median)	41-76 (Range)
Gender	Number	Percentage
M	31	70.5
F	13	29.5
Previous nephrectomy	Number	Percentage
Y	39	88.6
N	5	11.4
Antiangiogenic drug	Number	Percentage
Sunitinib	42	95.5
Sorafenib	1	2.3
Pazopanib	1	2.3
Prior line(s) of treatment	Number	Percentage
1	12	27.3
2	16	36.4
3	11	25.0
4	4	9.1
5	0	0
6	1	2.3
Prior treatment	Number	Percentage
IL-2	20	45.5
Avastin	13	29.5
Sorafenib	12	27.3
Pazopanib	2	4.5
Axitinib	3	6.8
mTOR inhibitor**	2	4.5
Clinical trial	9	20.5
Other	4	9.1

* Of the 39 patients who had prior Nx, 24 had cytoreductive nephrectomy. All patients were treated with VEGFR TKI for metastatic disease and not in the adjuvant or neoadjuvant setting.

** Mammalian target of rapamycin.

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