Stem Cells Increase in Numbers in Perinecrotic Areas in Human Renal Cancer

Mariana Varna1,2, Guillaume Gapihan1,2, Jean-Paul Feugeas1, Philippe Ratajczak1,2, Sophie Tan2, Irmine Ferreira1,2, Christophe Leboeuf1,2, Niclas Setterblad1, Arnaud Duval1,2, Jérôme Verine1,2, Stéphane Germain3,4,5,6,7, Pierre Mongiat-Artus1,2,8, Anne Janin1,2,3, and Guihlhem Bousquet1,2

Abstract

Purpose: Developing strategies to overcome resistance to sunitinib is a major challenge in human renal cell carcinoma (RCC). We hypothesized that sunitinib-induced tumor necrosis–associated hypoxia could interact with renal cancer stem cells in patients with metastatic RCC.

Experimental Design: We studied tissue samples from 7 patients with primary metastatic RCC, before and after sunitinib treatment, and from six xenograft models derived from human RCC. Two xenograft models were responders to sunitinib, the four others were nonresponders. CD133/CXCR4–coexpressing cells derived from the two responder xenograft models were used for in vitro studies.

Results: In the seven primary RCCs, we identified a significantly larger number of CD133/CXCR4–coexpressing cells in perinecrotic versus perivascular areas. Their numbers also significantly increased after treatment, in perinecrotic areas. We reproduced these clinical and pathologic results in all six RCC xenograft models with again a preferential perinecrotic distribution of CD133-expressing cells. Necrosis occurred at day 7 in the two responder models treated with sunitinib, whereas it occurred at day 21 in the untreated controls and in the four nonresponder models. Strikingly, when we studied the six RCC xenograft models at the time necrosis, whether spontaneous or sunitinib-induced, occurred, necrosis area correlated with stem-cell number in all 120 xenografted RCCs. When studied under experimental hypoxia, the number of CD133/CXCR4–coexpressing cells and their tumorigenic potency increased whereas their sensitivity to sunitinib decreased.

Conclusions: In human RCC, sunitinib was able to generate resistance to its own therapeutic effect via induced hypoxia in perinecrotic areas where cancer stem cells were found in increased numbers. Clin Cancer Res; 21(4); 916–24. ©2014 AACR.

Introduction

Antiangiogenic therapies have improved the prognosis of patients with metastatic renal cell carcinoma (RCC), but secondary resistance constantly occurs (1–3). These innovative therapies act through hypoxia-induced tumor necrosis (4), necrosis being considered an indirect marker of oxygen depletion (5).

Preclinical data suggest a central role of hypoxia in the resistance to tyrosine kinase inhibitors such as sunitinib (6, 7). Hypoxia induces resistance to chemotherapy in human cancer progenitors (8, 9), and to targeted therapies in chronic myeloid leukemia cells (10).

Hypoxia and necrosis occur in the spontaneous evolution of cancer. Hypoxia-inducible factor-1α (HIF1α) stimulates angiogenesis by target gene activation (11), and promotes endothelial cell progenitor and cancer stem-cell development (6, 12–14). In tissues, in which microenvironment conditions are thought to govern cancer stem cells, little is known about the role of hypoxia.

According to consensus criteria, cancer stem cells are able to self-renew and have tumorigenic potential (15). Different surface markers characterize them, including CD133, expressed by hematopoietic stem cells (16), renal progenitors (17), brain (18), and colon cancer stem cells (19), and CXCR4, expressed by human hematopoietic progenitors, renal progenitors (20), and pancreatic cancer stem cells (21, 22).

Using CD133 and CXCR4 on human primary RCC biopsies and xenografted samples, we identified stem cells around areas of spontaneous or sunitinib-induced necrosis. When these cells were sorted from xenografted human RCC before treatment, we demonstrated that hypoxia increased their tumorigenic potency, and decreased their sensitivity to sunitinib. Our results, therefore, strongly suggest that in human RCC, sunitinib was able to generate resistance to its own therapeutic effect via induced hypoxia in perinecrotic areas where cancer stem cells are found in increased numbers.

Materials and Methods

Patients and RCC samples

Samples were obtained from primary tumors of 7 patients with metastatic RCC, both before any medical treatment and after 3...
Translational Relevance

Developing strategies to overcome resistance to sunitinib is a major challenge in human renal cell carcinoma (RCC). Antiangiogenic therapies act through hypoxia-induced tumor necrosis, but the mechanisms by which hypoxia induces treatment resistance in tumors remain unknown. In primary RCC from patients with metastatic cancer, we identified CD133/CXCR4–coexpressing cells in perinecrotic areas. We reproduced this preferential distribution in six xenograft models established from tumor samples from patients with metastatic RCC. In two models that were responders to sunitinib, we showed that sunitinib-induced hypoxia increased the number of perinecrotic cancer stem cells, and that these cells had a lower sensitivity to the drug. The clinical relevance is high because resistance to antiangiogenic drugs is challenging daily oncology practice. Important data are added to existing knowledge about cancer treatment with antiangiogenic drugs because it opens a new field of pharmacologic research based on the fact that an antiangiogenic drug, initially efficient on a metastatic cancer, is also able to induce resistance to its own therapeutic effect.

months of sunitinib treatment (Pfizer) at 50 mg/d with a 4-week on and 2-week off schedule (1), sunitinib being the first line of treatment. Imaging-guided pretreatment biopsies were similarly performed at a minimum distance of 1 cm from necrotic areas detected on computed tomography, and processed as described in Supplementary Methods. Table 1 shows tumor characteristics.

In compliance with French Bioethics law (2004-800; June 8, 2004), all patients had been informed of the research use of the part of their samples remaining after diagnosis had been established, and did not oppose. Informed consent was obtained for each patient. The study was approved by the University Board Ethics Committee.

Human RCC xenografts

Six xenograft models of human RCC in nude mice were studied (see Supplementary Methods). The characteristics of the six xenografted human RCC models are shown in Table 2.

At day 21, when mice treated with sunitinib were compared with untreated mice, the xenograft model was considered as “responder” to sunitinib if tumor volumes were significantly smaller ($P < 0.01$).

### Table 1. Primary tumor characteristics

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Fuhrman grade</th>
<th>VHL status</th>
<th>Initial TNM status</th>
<th>Type of metastasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>RCC1</td>
<td>3</td>
<td>Mutated</td>
<td>pT1aN1M1</td>
<td>Lung, bone</td>
</tr>
<tr>
<td>RCC2</td>
<td>4</td>
<td>Mutated</td>
<td>pT4NXM1</td>
<td>Lung, pancreas</td>
</tr>
<tr>
<td>RCC3</td>
<td>3</td>
<td>Mutated</td>
<td>pT3NxM1</td>
<td>Lung, brain</td>
</tr>
<tr>
<td>RCC4</td>
<td>1</td>
<td>Mutated</td>
<td>pT2NxM1</td>
<td>Lung</td>
</tr>
<tr>
<td>RCC5</td>
<td>3</td>
<td>Mutated</td>
<td>pT3aNxM1</td>
<td>Lung</td>
</tr>
<tr>
<td>RCC6</td>
<td>4</td>
<td>Mutated</td>
<td>pT2N2M1</td>
<td>Lymph node, lung</td>
</tr>
<tr>
<td>RCC7</td>
<td>3</td>
<td>Mutated</td>
<td>pT1N0M1</td>
<td>Lung, brain</td>
</tr>
</tbody>
</table>

Results

Stem cells in perinecrotic areas

In the primary tumor samples of the 7 patients with metastatic RCC, before treatment and after 3 months of sunitinib, we identified large cells expressing CD133. When counted independently from their location in the tumor, there was no significant change after treatment (from 2.4% to 3.7%, \( P = 0.1 \)). Interestingly, CD133-expressing cells were significantly more numerous in perinecrotic than in perivascular areas, in both untreated patients (4.7% vs. 1.3%, \( P < 0.01 \)) and treated patients (8.3% vs. 1.9%, \( P < 0.01 \)). In treated patients, CD133-expressing cells were significantly more numerous than in untreated patients but only in perinecrotic areas (8.3% vs. 4.7%, \( P < 0.05 \); Fig. 1A).

In perinecrotic areas in all patients, 84.5% ±2.5% of these cells coexpressed CD133 and CXCR4, suggesting a stem-cell phenotype. These CD133/CXCR4-coexpressing cells were significantly more numerous in treated patients compared with untreated patients (7.3% vs. 3.9% \( P < 0.05 \)). In addition, HIF1\(\alpha\), a marker of hypoxia, was coexpressed in 95% of CD133/CXCR4 cells (Fig. 1B).

Six xenograft models of human RCC in nude mice were studied, obtained from engraftment of RCC primary tumor samples from patients with metastatic RCC. Each of the six xenograft models was treated with sunitinib for 21 days. A model was considered as responder to sunitinib treatment if its tumor volume at day 21 was significantly smaller than the mean tumor volume among untreated mice (\( P < 0.01 \); Fig. 1A).

When we studied the six xenograft models, we again demonstrated that CD133-expressing cells were significantly more numerous in perinecrotic than in perivascular areas, in both treated and untreated tumors. In addition, when we compared untreated and treated mice, CD133-expressing cells were significantly more numerous in treated mice, but only in perinecrotic areas (\( P < 0.05 \); Fig. 2C and Supplementary Fig. S2).

These six preclinical models enabled us to perform in situ analyses on whole xenografted tumors. After removal of the whole tumor and realization of five representative full sections, the percentages of necrotic areas and of CD133-positive cells were assessed in the five different sections for each tumor (Fig. 2D). When we studied untreated and treated mice together, including responders and nonresponders to sunitinib (\( n = 120 \) mice), the necrosis ratio in tumor tissue sections was significantly related to the mean number of CD133-expressing cells (\( P < 0.01 \); Fig. 2D).

When we used pimonidazole, another hypoxic marker, we showed that hypoxic living cells stained for pimonidazole were distributed around necrotic areas. This was observed in the RCC xenograft models when necrosis occurred, at day 21 for one untreated RCC xenograft model, and at day 7 under treatment for one xenograft model that was responder to sunitinib (Supplementary Fig. S3).

Because tissue necrosis is directly related to hypoxia, these results strongly suggest a link between hypoxia, whether sunitinib-induced or spontaneous, and an increased number of RCC stem cells.

CD133/CXCR4 stem cells had tumorigenic potential

To determine whether CD133/CXCR4-coexpressing cells were tumorigenic in vivo, we grew spheres from untreated RCC xenografts. When these spheres were cultured in normoxic conditions, confocal microscopy identified CD133/CXCR4-coexpressing cells (Fig. 3A).

These cells isolated from normoxic spheres induced tumor growth 3 weeks after injection of 2.105 cells into nude mice. This tumorigenic potential was observed with CD133/CXCR4–coexpressing cells from normoxic spheres derived both from the two sunitinib responder models (Fig. 3B) and the four nonresponder models (Fig. 4A).

As controls, 2.105 cells from the CD133/CXCR4 double-negative fraction of normoxic spheres injected in nude mice did not induce any tumor growth over a period of 2 months (Supplementary Table). Microscope analyses and cell counts showed that tumors that developed from CD133/CXCR4–coexpressing cells reproduced the initial primary human RCC features for morphology, cell proliferation, microvessel density, and stem-cell density (Figs. 3B and 4A).

Experimental hypoxia increased stem-cell tumorigenicity

We compared the tumorigenic potential of spheres cultured under hypoxic and normoxic conditions. After injection of CD133/CXCR4–coexpressing cells sorted from spheres, there

### Table 2. Characteristics of the six xenografted tumor models

<table>
<thead>
<tr>
<th>Tumor model</th>
<th>Pretreatment biopsy</th>
<th>Posttreatment surgery</th>
<th>Fuhrman grade</th>
<th>VHL status</th>
<th>Initial TNM* status at time of graft</th>
<th>Metastases</th>
<th>Responder to sunitinib</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRC1</td>
<td>+</td>
<td>3</td>
<td>Mutated</td>
<td>pT1aNxM1</td>
<td>Lung, bone</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>HRC2</td>
<td>+</td>
<td>2</td>
<td>Mutated</td>
<td>pT1aNxM1</td>
<td>Lung</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>HRC3</td>
<td>-</td>
<td>4</td>
<td>Wild-type</td>
<td>pT3aNxM1</td>
<td>Bone</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>HRC4</td>
<td>+</td>
<td>4</td>
<td>Mutated</td>
<td>pT4aNxM1</td>
<td>Lung</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>HRC5</td>
<td>+</td>
<td>4</td>
<td>Mutated</td>
<td>pT1bNxM1</td>
<td>Lymph node, lung</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>HRC6</td>
<td>+</td>
<td>4</td>
<td>Mutated</td>
<td>pT1bNxM1</td>
<td>Lymph node, lung</td>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>

was a significantly larger mean tumor volume after injection of hypoxic stem cells rather than normoxic stem cells ($P < 0.05$; Figs. 3C and 4B). This difference was significant from 42 days on for the two responder models (Fig. 3C) and for the four nonresponder models (Fig. 4B). To rule out a difference due to proliferation or angiogenesis, we counted Ki67- and CD31-expressing cells in tumor sections, and no significant difference was found in tumors grown from normoxic and hypoxic spheres (Fig. 3C).

When we tested the tumorigenic potential of serial dilutions of isolated CD133/CXCR4 cells from normoxic and hypoxic spheres, a significant increase in tumor growth occurred after injection of $2 \times 10^2$ and $2 \times 10^3$ cells when hypoxic spheres were compared with normoxic spheres. The difference was not significant after injection of a larger number of cells ($2 \times 10^4$ cells; Figs. 3C and 4C).

Experimental hypoxia increased the number of stem cells and decreased their sensitivity to sunitinib.

To assess the role of experimental hypoxia on the number of renal cancer stem cells, spheres derived from untreated tumors of the six human RCC xenograft models were cultured under hypoxic conditions.

After 5 days of experimental hypoxia, FACS analyses showed a significantly larger number of CD133/CXCR4 cells in hypoxic spheres compared with normoxic spheres ($P < 0.01$). This was found in the two responder models ($7.6\% \pm 2\%$ vs. $2.1\% \pm 0.7\%; P < 0.01$; Fig. 3D), and the four nonresponder models ($9.2\% \pm 2.2\%$ vs. $4.6\% \pm 1.1\%; P < 0.01$; Fig. 4C).

To assess sunitinib effects on RCC stem cells, we then analyzed whether CD133/CXCR4 cells from hypoxic spheres were more sunitinib resistant than CD133/CXCR4 cells from normoxic spheres, using an in vitro cytotoxicity assay. The IC$_{50}$ value of
Sunitinib was higher for hypoxic than for normoxic cells for both the two responder models (Fig. 3D), and the four nonresponder models (HRCC9 to HRCC12).

Discussion

In patients with metastatic RCC who were responders to sunitinib, we identified CD133/CXCR4–coexpressing stem cells in perinecrotic areas, and showed that their numbers increased after sunitinib administration. To our knowledge, this has never been reported in human RCC.

We chose CD133 and CXCR4 because they are two well-known markers of stem cells (16–20). In a series of 240 RCC, more than 20% positive cells were found for CD133-expressing cells in 22.5% of RCC, and for CXCR4-expressing cells in 60.8% of RCC (23), and high CXCR4 expression was predictive of poor response to sunitinib (24). The 7 patients we studied had at least a stable disease under sunitinib treatment (for this reason they underwent nephrectomy after treatment), and when we assessed the number of CD133/CXCR4–coexpressing cells, we found a mean percentage from 4.2% to 7.7%.

To further study these stem cells, we xenografted human RCC in nude mice, and performed a sequential study in untreated and sunitinib-treated mice. We obtained six models, two of which were responders to sunitinib (HRCC1 and HRCC8), and four nonresponders (HRCC9 to HRCC12).

Varna et al.
Figure 3.
In the two responder models, hypoxia increased the number of stem cells, their tumorigenicity and resistance to sunitinib. A, under normoxic conditions, confocal microscopy (Zeiss LSM510) shows that RCC sphere cells coexpress the stem cell markers CD133 and CXCR4. B, when grafted subcutaneously, CD133/CXCR4-coexpressing cells isolated by magnetic sorting from HRCC1 or HRCC8 spheres induce tumor growth in two groups of 5 nude mice. At day 63, microscope analyses show that tumors developed from CD133/CXCR4-coexpressing cells reproduce the initial RCC features in terms of proliferation (Ki67-positive cell count), microvessel density (CD31-positive cell count), and stem-cell density (CD133/CXCR4 cell count). C, tumor growth following s.c. injection of 2.10^3 CD133/CXCR4 cells obtained from HRCC1 or HRCC8 spheres cultured either under hypoxic or under normoxic conditions is significantly different (permutation test, P < 10^-5). At day 49, tumor growth for hypoxic cells is larger than for normoxic cells (n = 10 mice in each group); **, P < 0.01. Microscope analyses show that the differences in growth observed between xenografted tumors developed from hypoxic and normoxic spheres do not result from differences between numbers of Ki67- and CD31-positive cells. Follow-up of tumor growth after s.c. injection of serial dilutions of CD133^+CXCR4^+ cells isolated from hypoxic and normoxic spheres shows that only hypoxic cells are able to induce tumor growth at the minimal dilution of 2.10^2 cells, and that they induce a significantly larger tumor growth than normoxic cells at the dilution of 2.10^3 cells (n = 5 mice in each group); **, P < 0.05. D, Flow-cytometry analyses of HRCC1 and HRCC8 spheres cultured under normoxic and hypoxic conditions show significantly larger numbers of cells expressing CXCR4, and double-positive CD133/CXCR4 cells in hypoxic spheres; **, P < 0.05; ***, P < 0.01. MTT assays show that double-positive CD133/CXCR4 cells sorted from hypoxic spheres are more resistant to sunitinib than CD133/CXCR4 cells sorted from normoxic spheres in the two responder models; **, P < 0.01.
increased the number of CD133/CXCR4-coexpressing stem cells with a preferential perinecrotic distribution. When all xenografted human RCC, whether treated or untreated, responders or non-responders to sunitinib, were considered (i.e., 120 tumor samples), we demonstrated that the number of CD133/CXCR4-coexpressing cells was related to the extent of tissue necrosis. In a preclinical model of breast cancer cell lines (7), the number of cancer stem cells increased under sunitinib via hypoxia. Here, we studied renal and not breast cancer, and human tumor samples and not cancer cell lines.

Figure 4. In the four nonresponder models, hypoxia also increased tumorigenicity of stem cells and their resistance to sunitinib. A, when grafted subcutaneously, CD133/CXCR4-coexpressing cells isolated by magnetic sorting from HRCC9, HRCC10, HRCC11, or HRCC12 spheres induce tumor growth in four groups of 5 nude mice each. At day 56, microscope analyses show that tumors developed from CD133/CXCR4-coexpressing cells from each of the four nonresponder models reproduce the initial RCC features in terms of proliferation (Ki67-positive cell count), microvessel density (CD31-positive cell count), and stem-cell density (CD133/CXCR4 cell count). B, an s.c. injection of 2.10⁵ CD133/CXCR4 cells from spheres cultured either under hypoxic or under normoxic conditions shows that tumor growth for each of the four nonresponder models is significantly different (permutation test, \( P < 10^{-10} \)). At day 49, tumor growth for hypoxic cells is larger than for normoxic cells (\( n = 10 \) mice in each group); \( * P < 0.05 \); \( ** P < 0.01 \). C, follow-up of tumor growth after s.c. injection of serial dilutions of cells isolated from hypoxic and normoxic spheres shows that only hypoxic cells are able to induce tumor growth at the minimal dilution of 2.10² cells, and that they induce a significantly larger tumor growth than normoxic cells at the dilution of 2.10³ cells (\( n = 5 \) mice in each group); \( * P < 0.05 \); \( ** P < 0.01 \). D, MTT assays show that double-positive CD133/CXCR4 cells sorted from hypoxic HRCC9, HRCC10, HRCC11, or HRCC12 spheres sorted from the corresponding models; \( * P < 0.05 \). Flow-cytometry analyses of HRCC9, HRCC10, HRCC11, and HRCC12 spheres (pooled data) cultured under normoxic and hypoxic conditions show significantly larger numbers of cells expressing CXCR4, and double-positive CD133/CXCR4 cells in hypoxic spheres; \( P < 0.05 \); \( ** P < 0.01 \). MTT assays show that double-positive CD133/CXCR4 cells sorted from hypoxic HRCC9, HRCC10, HRCC11, or HRCC12 spheres are more resistant to sunitinib than CD133/CXCR4 cells sorted from normoxic spheres sorted from the corresponding models; \( * P < 0.05 \).

Regarding renal cancer stem cells, CD105, more than CD133, has been recommended following studies using cells selected by flow cytometry (25). However, CD105 is not appropriate for studies in whole-tumor tissue sections, because it is also expressed by tumor endothelial cells (26) and carcinoma-associated fibroblasts (27).
VHL gene mutations are found in 70% of human RCC (28), as was the case in our patients and the xenografted RCC. When present, VHL gene mutations lead to HIF accumulation (29), including HIF1α, recently characterized as a tumor suppressor (30, 31), and HIF2α, characterized as an oncprotein (30). Because HIFs and necrosis are two indirect markers of oxygen depletion (5), for this study based on human cancer tissue we chose to use the extent of necrosis rather than the number of HIF1α- or HIF2α-expressing cells as markers of hypoxia.

After sorting from untreated xenografted human RCC, the CXCR4/CD133–coexpressing stem cells were able to form spheres and to induce tumors in mice, two characteristic features of cancer stem cells (15). We showed in vitro that experimental hypoxia increased both their number and their tumorigenicity. Hypoxia can increase the number of glioblastoma (12) and breast cancer (14, 22) stem cells, but we have demonstrated here for the first time that hypoxia can increase tumorigenic potential and resistance to sunitinib of cancer stem cells from untreated human RCC.

The question of additional hypoxia on RCC-bearing VHL mutation opens discussion of the relative importance of intrinsic and extrinsic hypoxia in RCC cells. It has recently been shown that clear-cell RCC can express low levels of HIF1α, particularly when chromosome 1q4 is deleted (30, 32). Therefore, it is possible that not all RCC cells have the same constitutive pseudohypoxic level. In our preclinical models, human xenografted RCCs were treated by sunitinib, which affects endothelial cells and induces necrosis (4), and thus hypoxia in tumor cells through an extrinsic mechanism. In patients with metastatic RCC, resistance to antiangiogenic treatment is a major cause of poor survival (2, 3). In preclinical studies, it has been shown that resistance to sunitinib occurred because cancer cells acquired metastatic potential (33, 34), and that sunitinib mainly acts by vascular effect and induction of necrosis (4). Our demonstration that hypoxia increases the number of cancer stem cells while decreasing their sensitivity to sunitinib implies that the drug, by way of its initial effect on the tumor microvessels, is able to induce mechanisms of resistance to its own effect. This is coherent with the clinical observation showing that the benefit of sunitinib is only transitory (2).

Designing new strategies to optimize antiangiogenic therapies needs to take into account the deleterious effect of therapy-induced hypoxia, which, as shown in this study, is responsible for the emergence of cancer stem cells in RCC. Targeting cancer stem cells might, thus, be a promising future research approach to prevent acquired resistance to sunitinib. Molecules targeting endothelial cell progenitors such as TRC105, an antiendoglin antibody, are under development in metastatic RCC (35), but the effect of therapeutic antibodies targeting CXCR4 and/or CD133 on RCC cancer stem cells remains unknown.

The clinical relevance of our results is a major strength of this work, as resistance to antiangiogenic drugs is challenging daily practice in oncology. In addition, this study is to our knowledge the first to be performed on human RCC samples before and after treatment, the relevant results being reproduced in xenograft models responding to sunitinib treatment, thus generalizing the conclusions obtained in the patient samples.

Important data are added to existing knowledge about cancer treatment with antiangiogenic drugs because it opens a new field of pharmacologic research based on the fact that an antiangiogenic drug, initially efficient on a metastatic cancer, is also able to induce resistance to its own therapeutic effect.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Authors’ Contributions**

Conception and design: M. Varna, S. Germain, A. Janin, G. Bouquet

Development of methodology: A. Janin, G. Bouquet

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M. Varna, G. Gaphan, P. Ratajczak, C. Leboeuf, N. Setterblad, A. Duval, A. Janin, G. Bouquet

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J.-P. Feugeas, C. Leboeuf, G. Bouquet

Writing, review, and/or revision of the manuscript: M. Varna, J. Venne, P. Mongiat-Artus, A. Janin, G. Bouquet

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M. Varna, S. Tan, I. Ferreira

Study supervision: P. Mongiat-Artus, G. Bouquet

**Grant Support**

This work was supported by University-Paris-Diderot, INSERM, ANR, and Canceropole-INCa.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received March 21, 2014; revised December 3, 2014; accepted December 6, 2014; published OnlineFirst December 11, 2014.


Clinical Cancer Research

Stem Cells Increase in Numbers in Perinecrotic Areas in Human Renal Cancer

Mariana Varna, Guillaume Gapihan, Jean-Paul Feugeas, et al.


Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-14-0666

Supplementary Material
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2014/12/12/1078-0432.CCR-14-0666.DC1

Cited articles
This article cites 34 articles, 13 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/21/4/916.full.html#ref-list-1

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.