Anti-angiogenic drugs have proven to be efficient in most types of cancers, but secondary resistance is constantly observed, and mechanisms of resistance remain poorly understood in patients. In studies on human samples of renal cell carcinoma, our team recently demonstrated that sunitinib, a tyrosine kinase inhibitor, was able to generate resistance to its own therapeutic effect in cancer stem cells via induced hypoxia. This review highlights the main perspectives for innovative therapeutic strategies to overcome acquired resistance to anti-angiogenic drugs: i) Should we adjust our protocols on the basis of the moment of onset of necrosis in the tumor? ii) Using hyperoxia to decrease the number of cancer stem-cells in the resistant metastasis and resensitize the tumor could be another innovative therapeutic perspective. iii) Why not directly target cancer stem-cells? Although many questions remain, the results of our study support cancer stem-cell evaluation in biopsies of patients treated with sunitinib, and further research on the role of hypoxia in tumors resistant to anti-angiogenic drugs.

Keywords: cancer stem-cell; anti-angiogenic drugs; drug resistance; hypoxia

To cite this article: Guilhem Bousquet, et al. How anti-angiogenic drugs are able to induce resistance to their own therapeutic effect in human metastatic renal carcinoma. Can Cell Microenviron 2015; 2: e669. doi: 10.14800/ccm.669.

This study is original because we first analyzed human tumor samples obtained before and after treatment with sunitinib from the same patients with metastatic renal cell carcinoma. We observed that renal cancer stem cells increased in numbers after treatment. We then reproduced this effect of sunitinib in xenograft models derived from human tumor samples of metastatic renal cell carcinoma. This is a major strength of the study, since these innovative models reproduce characteristics of human cancers more accurately than xenografts obtained from human cancer cell lines[10] and they are suitable for pharmacological pre-clinical studies [11]. Until now, similar conclusions have been
obtained only from pre-clinical models using breast cancer cell lines [12-13], or glioma cell lines [14].

Thus, using these patient-derived renal cell carcinoma xenografts, we have shown that the number of renal cancer stem cell is directly linked to the percentage of necrosis in the tumor. This result was found for both spontaneous and sunitinib-induced necrosis. In pre-clinical studies, it has been shown that tumor necrosis is induced by sunitinib because the drug targets neo-angiogenic microvessels [11, 15]. In clinical settings, there is also radiological evidence of necrosis induced by anti-angiogenic drugs in patients with metastatic renal cell carcinoma. In addition, radiological criteria combining changes in tumor density and in tumor size [16-17] distinguish prognostic groups better than the standard criteria that only assess changes in tumor size [18].

As necrosis is an indirect marker of hypoxia, we decided to study the in vitro effect of experimental hypoxia on renal cancer stem cells sorted from these renal cell carcinoma xenografts. We have thus shown that experimental hypoxia increased their resistance to sunitinib.

Our results are in accordance with the clinical observation that in patients treated with sunitinib for metastatic renal cell carcinoma, an initial control of the tumor is constantly followed with a tumor re-growth after a median time of 11 months [8].

The findings of our study highlight a possible explanation for resistance to anti-angiogenic drugs. Sunitinib, by way of its main effect on endothelial tumor cells, increases the number of renal cancer stem cells and could then contribute to its own resistance. Since neo-angiogenesis is a pathological process common to all tumors, targeting efficiently tumor vessels can lead to an increase in numbers of cancer stem cells, whatever the tumor type.

Our results open fields for future research in the area of cancer stem cells in order to decipher their intrinsic mechanisms of resistance to hypoxia at the tissue level.

Our results also open perspectives for innovative therapeutic strategies to overcome acquired resistance to anti-angiogenic drugs, by tailoring protocols for the treatment of metastatic renal cell carcinoma:

1) Should we adjust our protocols on the basis of the moment of onset of necrosis in the tumor? A sequential combination of an efficient anti-angiogenic treatment followed by focal destruction of partially necrotic residual metastasis before re-growth occurs (surgery, radiotherapy, cryoablation, radiofrequency) could easily be implemented in daily clinical practice.

This also raises the question of large necrotic untreated tumors: shall we start by reducing tumor volume and then prescribe anti-angiogenic drugs?

2) Using hyperoxia to decrease the number of cancer stem-cells in the resistant metastasis and resensitize the tumor could be another innovative therapeutic perspective.

In vitro, it has been shown that hyperoxia restores sensitivity to drugs in chemoresistant glioblastoma cells [19]. In a pre-clinical rat model of breast carcinoma, hyperbaric oxygen treatment induced mesenchymal-to-epithelial transition of tumor cells [20], probably restoring a more differentiated phenotype.

3) Why not directly target cancer stem-cells?

A major difficulty in targeting cancer stem-cells is the absence of specificity of surface markers, and thus a major risk of adverse events from direct targeting of normal cells. Although most current data are in vitro and in vivo pre-clinical data, there are some interesting preliminary results obtained in patients with malignant tumors.

Thus, by selective targeting of leukemia-initiating cells in adult T-cell leukemia/lymphoma (ATL) [23], arsenic-based treatment gave promising signs of efficiency in patients with disease refractory to standard treatment [24].

In another model, Schlaak et al. recently reported the case of a patient with metastatic melanoma refractory to two lines of chemotherapy. They successfully treated him with a combination of a cytotoxic agent and an anti-CD20 monoclonal humanized antibody to eradicate the sub-population of CD20-expressing cells with stemness characteristics in the melanoma metastases of this patient [25].

Although many questions remain, the results of our study support cancer stem-cell evaluation in biopsies of patients treated with sunitinib, and further research on the role of hypoxia in tumors resistant to anti-angiogenic drugs.

Conflicting interests

The authors have declared that no competing interests exist.

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