Histological and immunohistochemical characterization of Sunitinib efficacy on A498 human renal cell carcinoma xenografts in athymic nude CD-1 female mice

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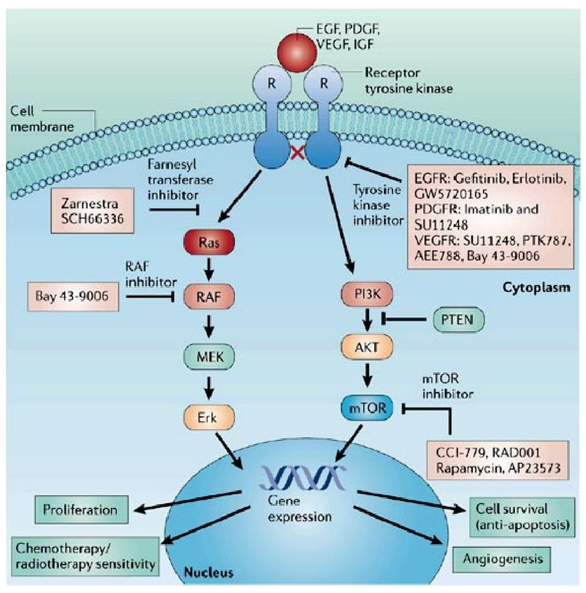
INTRODUCTION

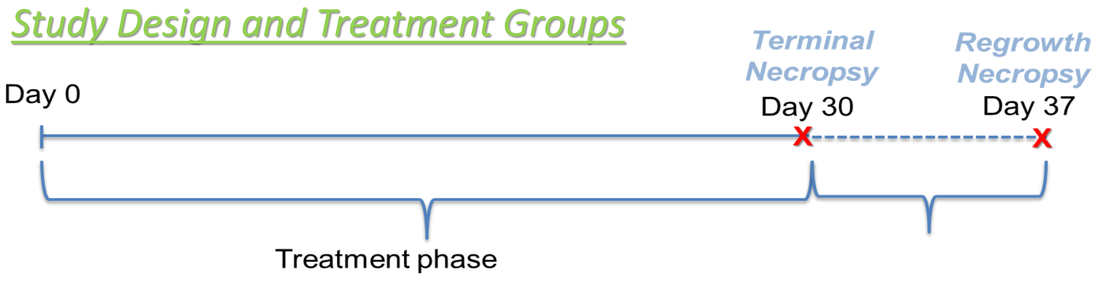
- Sunitinib is a marketed small-molecule (Sutent®, Pfizer) that has shown clinical efficacy in the treatment of advanced renal cell carcinoma.
- Sunitinib inhibits the tyrosine kinase activity of vascular endothelial growth factor receptors (VEGFR-1, VEGFR-2, and VEGFR-3), platelet-derived growth factor receptors (PDGFR- α and PDGFR-β) and stem cell growth factor receptor KIT (CD117) (Huang et al, 2010).
- Antiangiogenic, proapoptotic and antimitotic effects of sunitinib (Hong at al, 2009) have also been demonstrated.
- The objective of this study was to assess the efficacy of sunitinib on the subcutaneous growth of A498 human renal cell carcinoma in female nu/nu (Crl:CD1-*Foxn1*^{nu}) mice.



In addition, the methodology applied herein provides a possible example of histological and immunohistochemical characterization of antineoplastic and antiangiogenic drug efficacy in tumor xenografts.

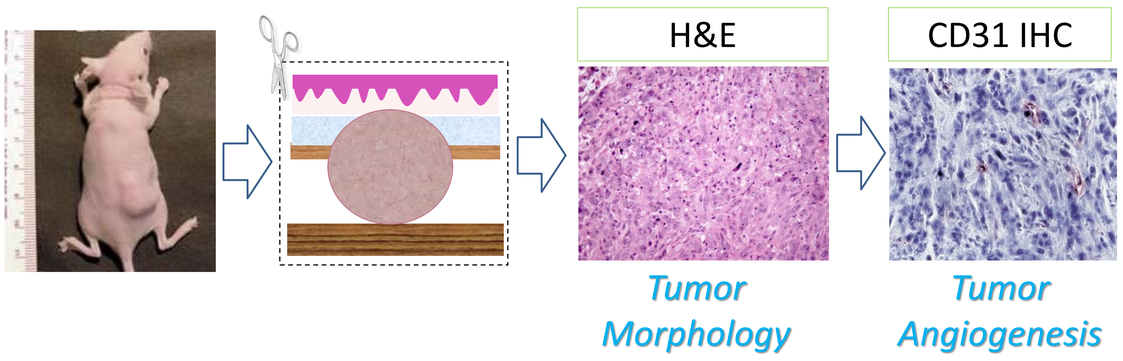
MATERIALS AND METHODS

Study Design and Treatment Groups



Sunitinib was subcutaneously administered once a day by oral gavage to athymic nude female CD-1 mice injected with A498 cells at doses of 0, 20, 40, or 80 mg/kg/day.

Histopathology investigations

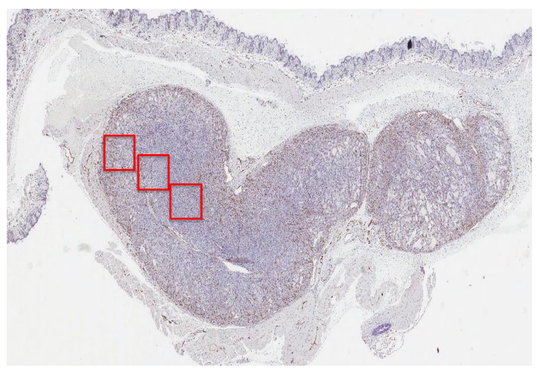


H&E: Tumor Morphology

Tumor Morphology	
Tumor size (area)	Aperio Imagescope Annotation tool
Stroma	0 = No stroma 1 = Scant amount of stroma, thin collagenous septa 2 = Mild amount of stroma, thin collagenous septa with vascular lacunae lined by endothelial cells 3 = Moderate amount of stroma, broader collagenous septa, vascular lacunae lined by endothelial cells; the stroma multifocally dissects the mass
Intratumoral necrosis	0 = No necrosis 1 = ≤ 20% of the xenograft extension 2 = between 20% to 50% of the xenograft extension 3 = between 50% to 80% of the xenograft extension 4 = ≥ 80% of the xenograft extension
Mitotic Index (MI)	Mean number of mitoses in 10 randomly selected high power field (HPF = 400X) in viable tumor fields
Apoptotic Index (AI)	Mean number of apoptosis in 10 randomly selected high power field (HPF = 400X) in viable tumor fields

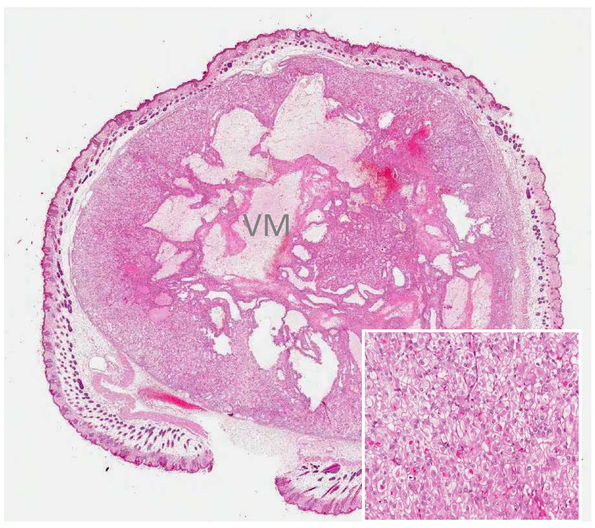
CD31 IHC: Tumor Vasculature

To evaluate the extent of tumor angiongenesis, 4 µm-thick sections for each tumor sample were immunostained with a primary rat monoclonal antibody against CD31 (clone SZ31 Dianova). Measurements of Endothelial Area (EA), corresponding to the surface of C31-positive endothelial structures, was performed using ImageJ analysis program (http://rsb.info.nih.gov/ij) in 3 200x microscopic fields, representative of the outer, intermediate and inner portions of the tumor.



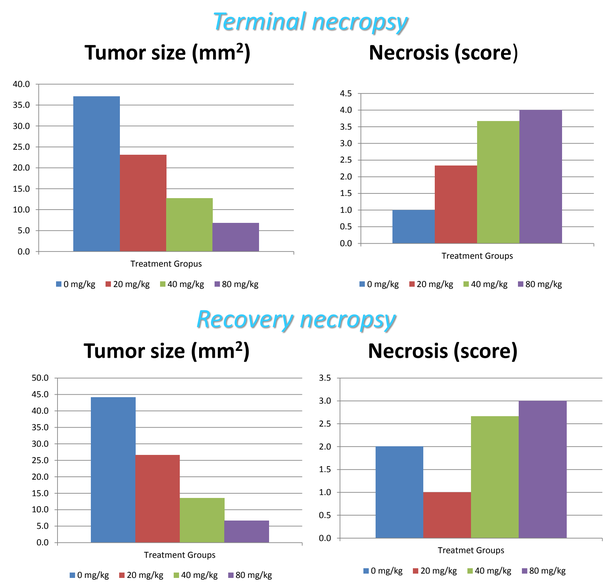
RESULTS

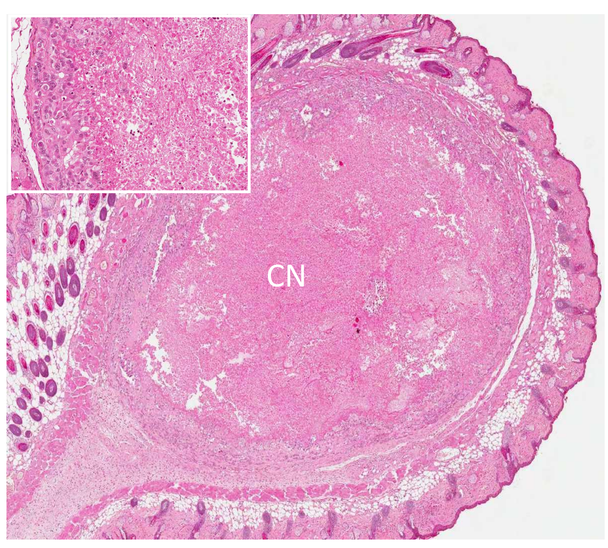
H&E: Tumor Morphology



Neoplastic cells were closely packed into tubuloacinar structures, separated by variable amount of fibrovascular stroma. In the central portion of the mass, tumor cells delimited irregularly sized and shaped channels, variably filled with blood cells (Vascular Mimicry, VM). Neoplastic cells were polygonal and characterized by clear ill-defined vacuoles (inset). Peripheral invasion was not observed.

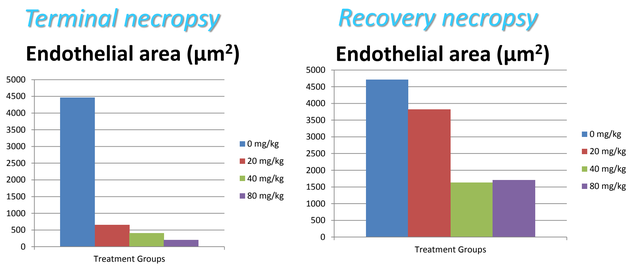
H&E: Sunitinib effects

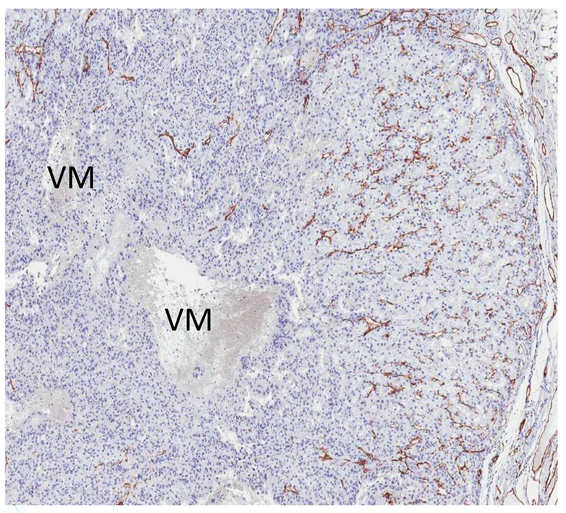




Sunitinb reduced the tumor area and increased the extent of intratumoral necrosis at the end of treatement period with a dose-related trend. Coagulative necrosis (CN) affected the core of the mass, even if a peripheral rim of surviving neoplastic cells was still observed (inset). After one week from terminal sacrifices, the tumor area in mice previously treated at 20 and 40 mg/kg/day was 15% or 6 % higher than the mice killed at term from the same dose groups; furthermore an increase in the MI and a decrease in the necrosis extent was observed at the same doses. Regrowth of the mass was observed only in one mouse previously treated at 80 mg/kg/day.

CD31: Sunitinib effects





At the end of 28-day treatment period, sunitinb-treated mice showed a dose-related decrease in the Endothelial Area (EA) up to 22.4 fold the control value. In the regrowth phase, the EA in mice previously treated at 20, 40 and 80 mg/kg/day was respectively 5.8, 4.1 and 8.6 fold higher than in mice killed at term from the same dose groups. CD31-positive (endothelial-lined) structures, representing tumor vasculature, were prominent along the capsule or at the periphery of the mass and progressively decreased toward the center. Tumor vasculature was characterized by linear, slit-like to circular profiles with frequent and irregular branches, separating cords and acini of neoplastic cells. At the center of the mass, vascular-like structures, lined by CD31-negative neoplastic cells (vascular mimicry, VM) were also evident.

DISCUSSION

The effects of sunitinib on the Endothelial Area were consistent with the described pharmacology: impaired angiogenesis and ingrowth of endothelial elements in treated A498 xenografts resulted in coagulative necrosis of the central, less perfused regions of the mass, that were more susceptible to ischemic injury; moreover, the regions of the tumors supplied by VM-channels were severely affected by treatment, confirming susceptibility of these complementary vascular structures to agents inhibiting VEGF pathway (Chen YS and Chen ZP, 2014).

A peripheral rim of surviving neoplastic cells was observed even in the most affected samples, allowing regrowth of the tumor mass and re-establishment of vascular network after treatment withdrawal.

References

Chen YS and Chen ZP. Vasculogenic mimicry: a novel target for glioma therapy. Chin J Cancer, 2014; 33(2):74-79.

Huang D, et al. Sunitinib acts primarily on tumor endothelium rather than tumor cells to inhibit the growth of renal cell carcinoma. Cancer Res. 2010; 70(3):1053-62.

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