Resistance to sunitinib in RCC: sequestration in lysosomes, inhibition of autophagic flux and inflammatory response

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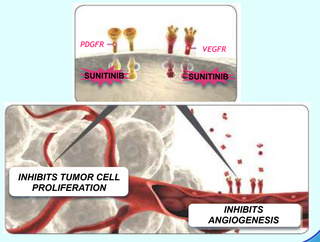
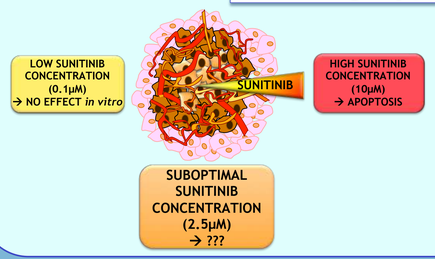
Introduction

Sunitinib — an oral tyrosine kinase inhibitor targeting in particular c-KIT, platelet-derived growth factor receptors α and β, vascular endothelial growth factor receptors 1, 2, and 3, is one of the first-line therapy for metastatic renal cell carcinoma (RCC).
Sunitinib prolongs progression-free survival in patients with metastatic RCC. However, in most of cases, patients relapse after one year of treatment.

➡ Resistance is an important clinical outcome, but its underlying mechanisms are largely unknown.



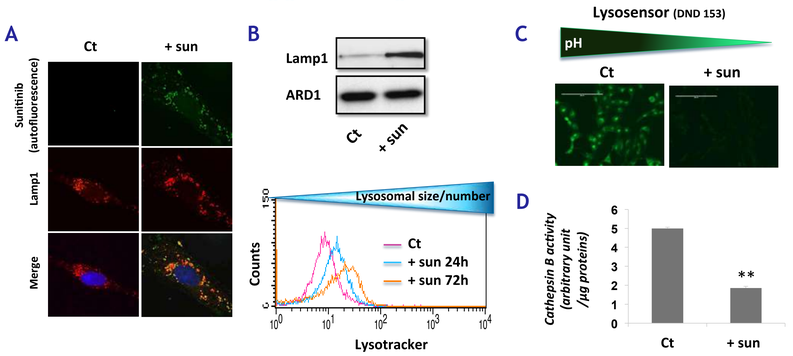
Working Hypothesis



Heterogenous distribution of sunitinib in the tumor is it responsible of RCC relapse ?

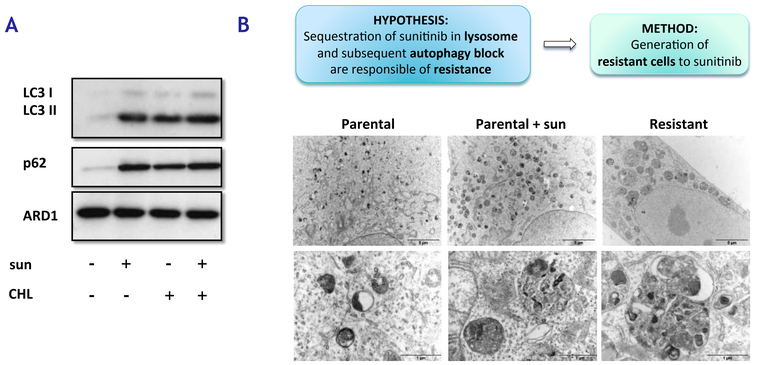
Results

FIG 1: Lysosomal sequestration of sunitinib triggers drug resistance



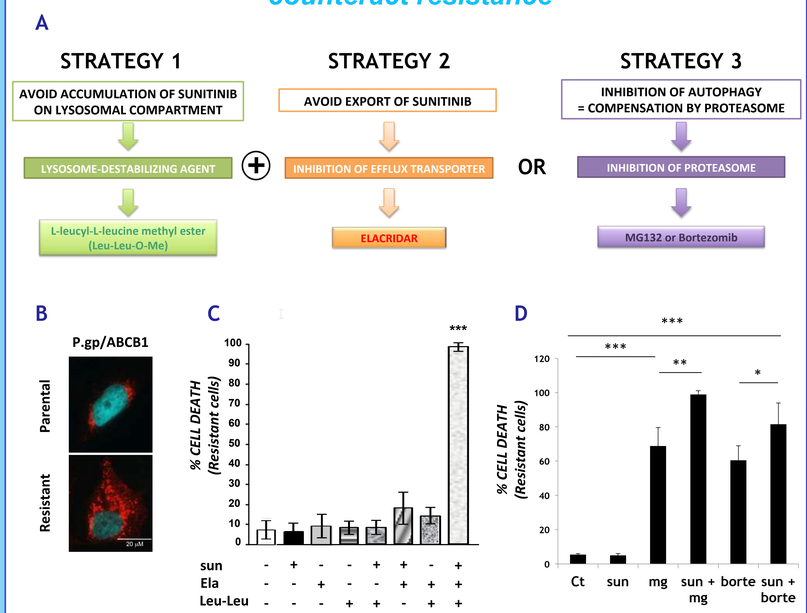
(A) Fluorescence microscopy revealed that sunitinib (Green) (sun, 2.5µM-24h) was specifically sequestered in lysosomal compartments (Red-Lamp1) of renal cells carcinoma (786-0-RCC).
(B) Sunitinib treatment presented an increase of lysosomal mass with an increase of Lamp1 expression and lysotracker labelling over time. ARD1 was used as a loading control.
(C) Fluorescence intensity of Lysosensor DND-153 (which detected an acidic pH) revealed that sunitinib (sun, 2.5µM-24h) disturbed the pH.
(D) Cathepsin B activity in organelle compartment (fractionment assay) decreased after sunitinib treatment.

FIG 2: Autophagy flux is impaired in sunitinib treated cells and Resistant cells



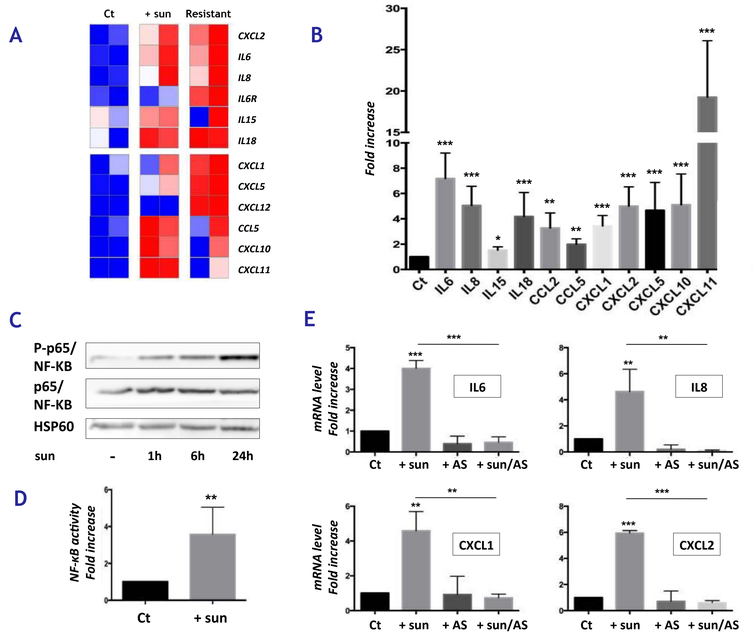
(A) RCC treated with sunitinib (sun, 2.5µM) with or without chloroquine (CHL, 20nM) during 24h presented an increase of LC3-II and p62 which is comparable in all conditions means that autophagic flux is impaired.
(B) Electronic microscopy analyses revealed that sunitinib resistant cells induce formation of bigger giant autolysosome compared to cells treated with sunitinib and even more compared to parental cells.

FIG 3: Impairment of lysosomal sequestration and export of sunitinib or proteasome inhibition counteract resistance



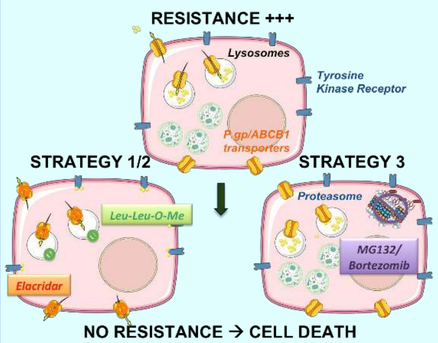
(A) Strategy against Resistant cells.
(B) Resistant cells presented an increase of P.gp ABCB1) expression, an ABC efflux transporter.
(C) Triple combination of sunitinib treatment with Elacridar (Ela, 5µM), an inhibitor of P.gp and ABCG2 transporters and Leu-Leu-O-Methyl (Leu-Leu, 1mM) induceed cell death of Resistant cells.
(D) MG132 (mg, 10µM) or Bortezomib (borte, 100µM), two inhibitors of proteasome, synergised with sunitinib to induce cell death of Resistant cells.

FIG 4: Sunitinib induces an inflammatory response



(A) RNAseq revealed an inflammatory response in sunitinib treated (sun, 2.5µM-48h) and Resistant cells.
(B) mRNA of different interleukins and chemokines are increased in RCC treated with sunitinib (sun, 2.5µM-48h). Quantification of mRNA level by RT-qPCR compared to control.
(C) Phosphorylation of p65 is increased in RCC treated with sunitinib (sun, 2.5µM).
(D) Luciferase assays revealed that sunitinib (sun, 2.5µM-48h) induced an increase of NF-κB activity.
(E) Inhibition of NF-KB by AS602858 (2.5µM) blocked the inflammatory response induced by sunitinib treatment. Quantification of IL6, IL8, CXCL1 and CXCL2 mRNA level by RT-qPCR at 48h.

Conclusion



- Lysosomal sequestration of sunitinib is a mechanism of drug resistance.
- Resistant cells stimulated the expression of P.gp/ABCB1, which participates in the accumulation of sunitinib in autolysosomes and favours its cellular efflux.
- Impairment of autophagy induces proteasome dependance.

Combination of Leu-Leu-O-Me and Elacridar or MG132/Bortezomib could represent a therapeutic strategy against sunitinib resistant RCC

- Sunitinib treatment induces an inflammatory response with increases in IL6, IL8, IL15, IL18, CCL2, CCL5, CXCL1, CXCL2, CXCL5, CXCL10 and CXCL11.
- NF-KB is induced and involved in the secretion of different chemokines and interleukins under sunitinib treatment.

Inflammatory mediators could participate in suntinib resistance by modulating parameters of the microenvironment