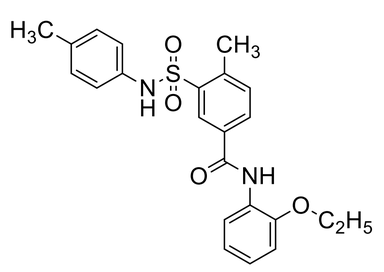
Functional evaluation of the new anti-cancer agent NRP-a308 on clear cell Renal Cell Carcinoma model expressing the different Neuropilin isoforms

A. Dumond *(1), R. Grépin (1), L. Demange (2), C. Ronco (2), G. Pagès (1,3), R. Benhida(2)*

(1) Centre Scientifique de Monaco, Biomedical Department, MONACO - (2) Institut de Chimie de Nice, NICE, FRANCE - (3) UMR 7284 INSERM U1081, IRCAN, NICE, FRANCE

Introduction

Clear cell Renal Cell Carcinoma (ccRCC) are among the most vascularized tumors. They represent a paradigm of tumor angiogenesis but also an excellent model to evaluate the efficacy of new anti-angiogenic agents. Sunitinib (Sutent®), the anti-angiogenic ccRCC reference treatment, induces a transient effect with resistance of most of the patients after a few months of treatment. Tumor cell dissemination via the lymphatic network observed in patients treated by sunitinib may be one cause of progression. In this context, Neuropilins (NRPs), co-receptors of VEGF receptors, have emerged as new relevant targets in oncology. Indeed, NRPs overexpression in patient tumors is correlated with a poor prognosis. NRPs downregulation by shRNA in ccRCC model cell lines results in decreasing cancer cells migration, invasion and tumor cells extravasation in the lymphatic network. Compound NRPa-308, a new NRP-1 antagonist, has recently been reported which exerts in vitro anti-angiogenic and anti-proliferative effects, and in vivo anti-cancer effects in mice xenografted with human aggressive breast cancer cells (MDA-MB-231). The work presented aims to demonstrate that NRP-a308 is an anti-cancer molecule able to target ccRCC cells expressing either NRP-1 or NRP-2, or both at the same time.

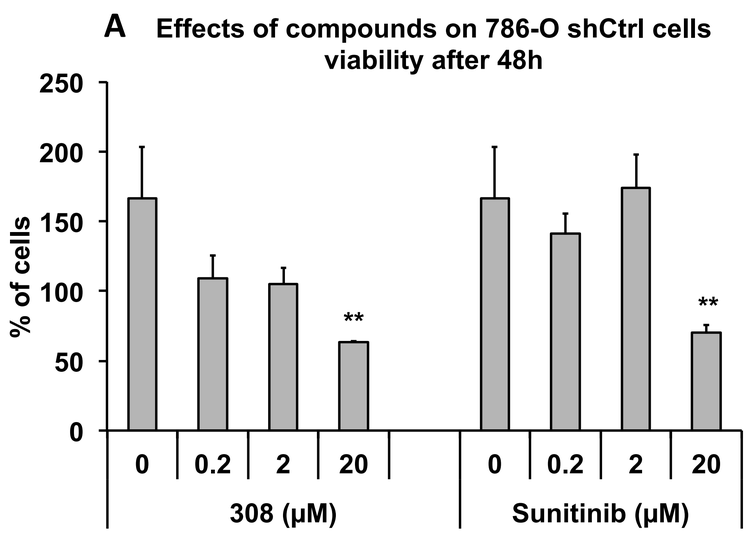


NRP-a308 structure

Methods and Results

Cell Proliferation

NRP-a308 reduces cell proliferation at 0.2µM after 48h, while sunitinib shows an efficient effect at higher concentrations (>2µM) *(figure 1A)*. NRP-a308 IC_{50} after 48h for 786-O shNRP cell lines are 10 times bellow those of sunitinib *(figure 1B)*.



B

IC_{50} after 48h (µM)	NRP-a308	Sunitinib
shCtrl	0.01	1.84
shNRP1	0.10	2.92
shNRP2	0.06	1.90

Figure 1 NRP-a308 is more efficient than sunitinib on ccRCC cell lines. A. Effects of treatments on 786-O cell proliferation after 48h evaluated by MTT assays. *B.* IC_{50} value after 48h of treatment on 786-O shNRP cell lines.

Cytotoxicity/Cytostaticity

After one week of treatment by NRP-a308 on 786-O shNRPs colonies, cytotoxic effects are observed at 0.2µM with less and smaller colonies. This cytotoxic effect is less important for sunitinib at 0.2µM *(figure 2)*.

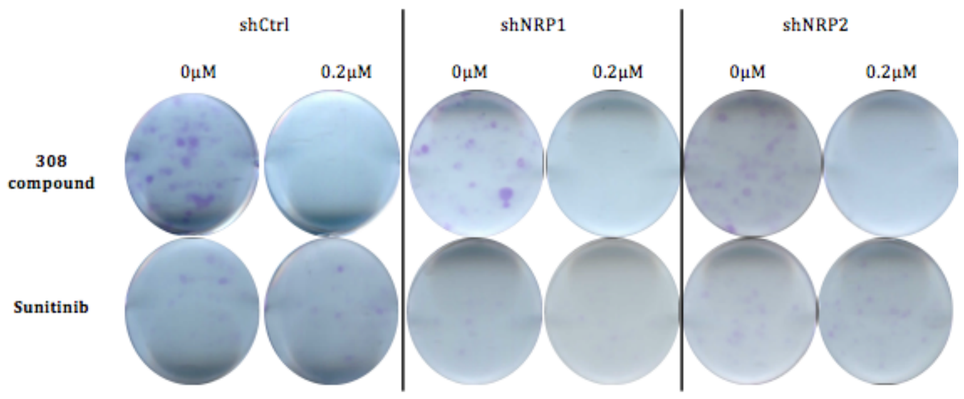


Figure 2. NRP-a308 prevents cell proliferation. Clonogenic assays. Coloration by Crystal Violet 0.01% after 1 week of treatment.

After removing treatments for one week, the number of colonies is still decreasing, an accumulation of the treatment in the cells may occurs *(figure 3)*.

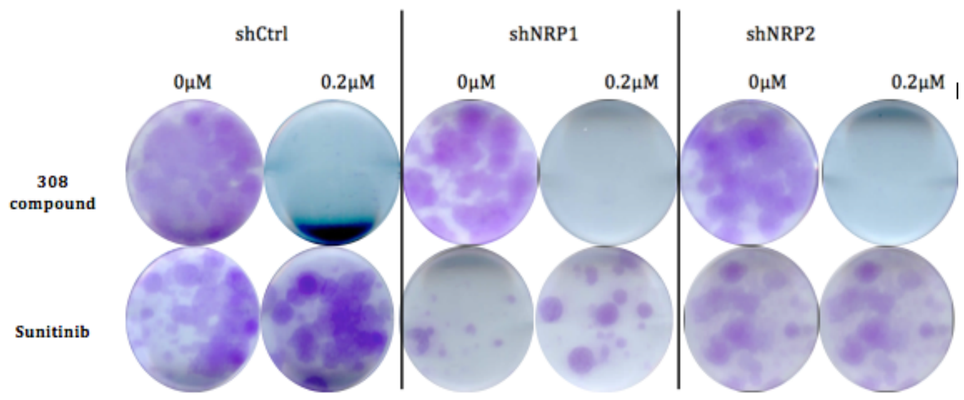


Figure 3. NRP-a308 exerts a cytotoxic effect. Clonogenic assays following one week on and one week off treatment. Coloration by Crystal violet 0.01%.

Cell migration

Cell migration was measured by Scratch assay *(figure 4)*.

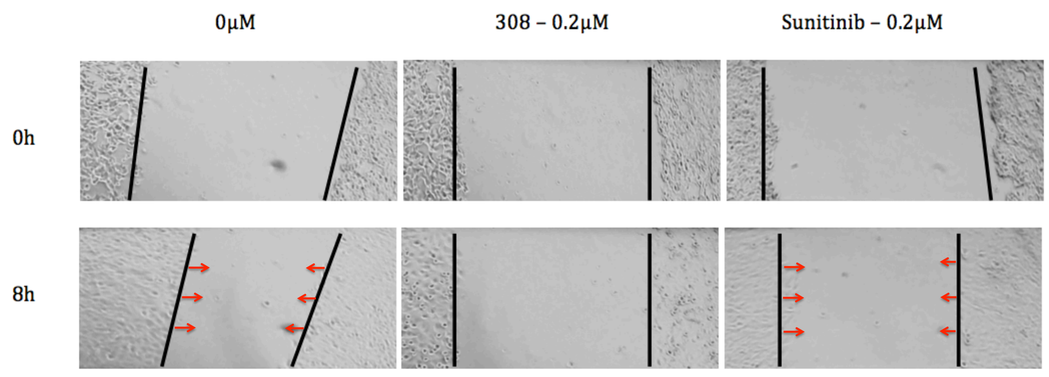


Figure 4. NRP-a308 inhibits cell migration. Images of the scratchs at 0h and after 8h of treatment.

NRP-a308 inhibits 786-O shNRPs cell migration and this through NRP-1 *(figure 5A)*. On the other side, sunitinib starts to have a small effect on cells migration at 2µM *(figure 5B)*.

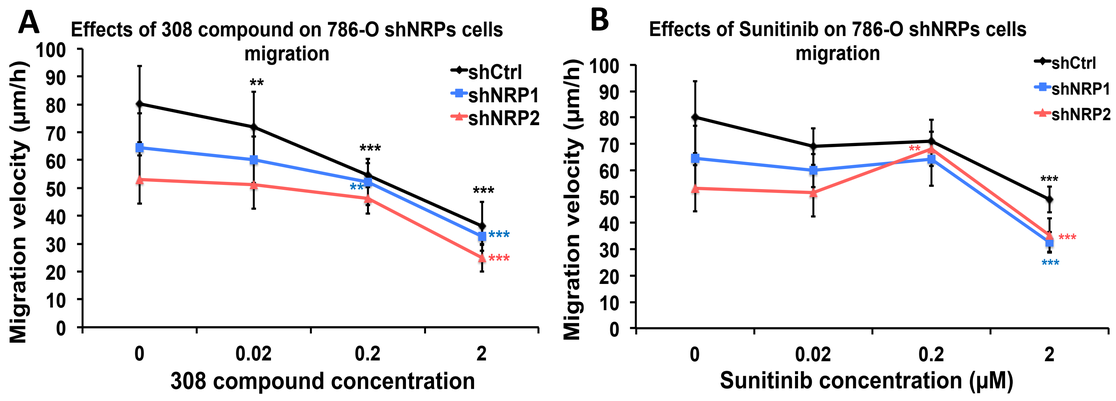


Figure 5. NRP-a308 inhibits 786-O cell migration through NRP-1. Effects of compounds on 786-O cell migration velocity. *A.* Effects of NRP-a308 on the different 786-O shNRPs cells line. *B.* Effects of sunitinib on the different 786-O shNRPs cells line.

This experiment shows also that migration is dependant of the expression of NRP-2 with a migration velocity inferior for shNRP2 compared to shCtrl 786-O cells.

Protein expression

NRP-a308 inhibits two proliferative and survival signalling pathways (ERK and AKT) in a more efficient manner than sunitinib.

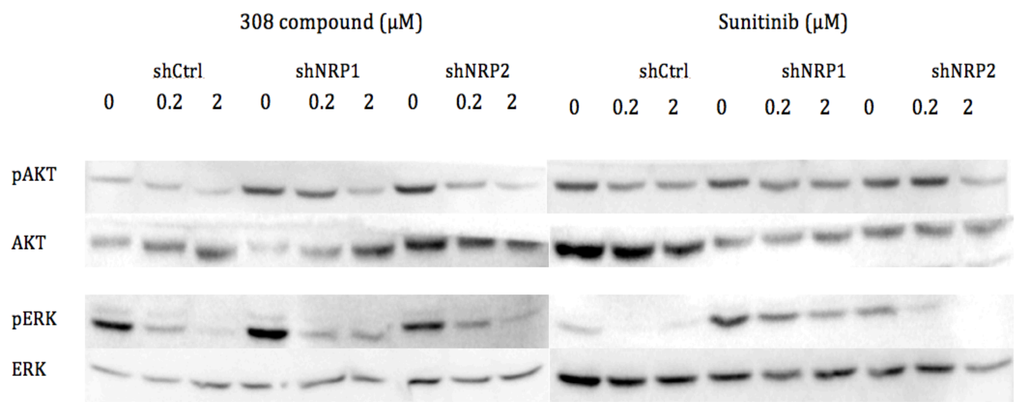


Figure 6. NRP-a308 inhibits proliferative and survival signalling pathways. Effects of compounds on ERK and AKT protein expression and activity after 10min of treatment evaluated by their phosphorylated forms by immunoblots.

CONCLUSION

These experiments have shown that NRP-a308 is a relevant compound to target ccRCC showing good efficiency in reducing 786-O shNRP cells viability, proliferation and migration. Furthermore, NRP-a308 is more efficient than sunitinib, ccRCC reference treatment, with IC50 more than 10 times lower. NRP-a308, initially described as a NRP-1 inhibitor, still has an effect on ccRCC even if NRP-1 expression is decreased. NRP-a308 is a promising inhibitor for ccRCC treatment but further investigation are needed to determine which others receptors are targeted.