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

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Abstract

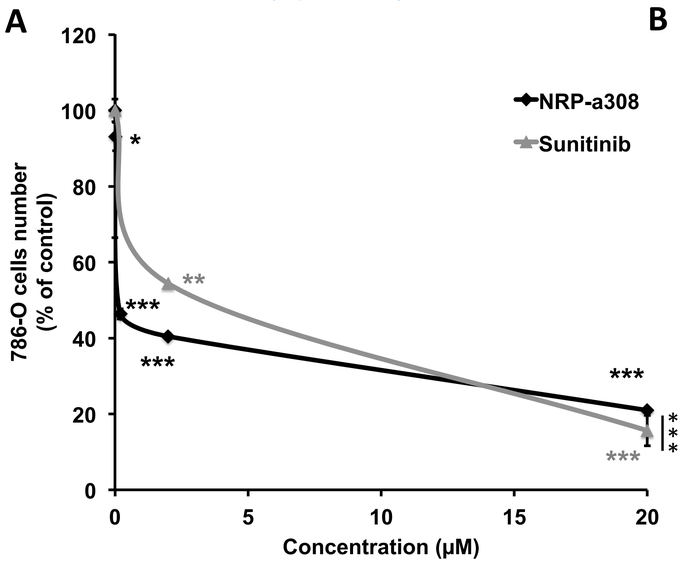
Anti-angiogenic therapies are used for the treatment of metastatic ccRCC (mccRCC). The current reference therapy in the first line is the multi-kinase inhibitor sunitinib (Sutent®). However, relapses appear after a few months. We recently describe that VEGF-C, the main pro-lymphangiogenic factor, represents one of the main actors of an evolutive disease with dissemination of tumour cells through the neo-formed VEGF-C dependent lymphatic network. These results highlight the urgent need to develop alternative therapeutic strategies for mccRCC at relapse on conventional treatment.
Moreover, a distinct family of VEGFs co-receptors, the Neuropilins (NRPs), has emerged as relevant oncology targets. NRPs form complexes with VEGFs and their receptors and induce cell migration, survival, and tumour growth. In ccRCC, inactivation of *NRP-1* by shRNA decreases cancer cell migration, invasion, and tumour growth. While *NRP-2* down-regulation results in decreased tumour cell extravasation in the lymphatic network, and in reduced metastatic dissemination. These results highlight the pivotal role of NRP-1 and NRP-2 in ccRCC aggressiveness. However, since gene inactivation mediated by shRNA remains a challenging therapeutic option, our attention was focused on small-sized NRPs antagonists. Thus, we developed a NRP antagonist (NRPa-308), which was previously shown to exert anti-cancer effects on human aggressive breast cancer. While down-regulation of NRP-1 by shRNA inhibited cell proliferation, decreased NRP-2 expression enhanced it. Moreover, cell migration was more importantly inhibited by NRP-2 than by NRP-1 knockdown. NRP-a308 inhibits ccRCC cell proliferation and migration more efficiently than sunitinib. Therefore, NRP-a308 may represent a new therapeutic tool by preventing tumour cell proliferation and invasion.

Methods and Results

Cell Viability

NRP-a308 reduces 786-O cell proliferation more efficiently than sunitinib *(figure 1A)*. NRP-a308 IC_{50} after 48h for 786-O shNRP cell lines are 10 times bellow those of sunitinib *(figure 1B)*.

A



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 cell proliferation after 48h evaluated by MTT assays. *B.* IC_{50} value after 48h of treatment on 786-O shNRP cell lines.

Cell migration

786-O cell migration is correlated with the expression of NRP-2. Indeed, down-regulation of NRP-2 in 786-O cells decreases cell migration velocity *(figure 4)*.

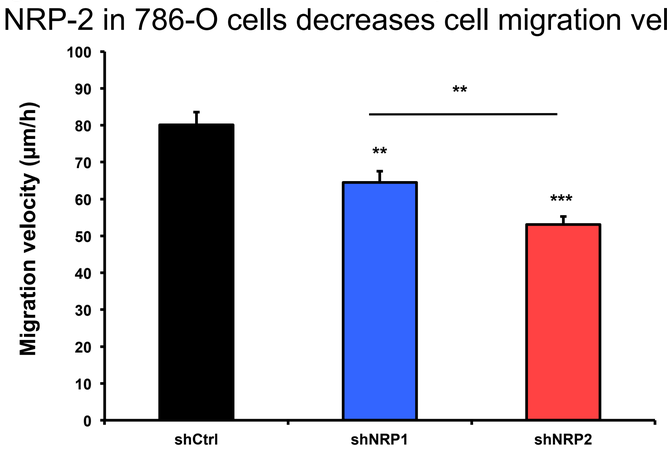


Figure 4. Migration velocity is dependant on NRP-2 expression. Effects of NRPs down-regulation on 786-O cell migration velocity measured by scratch assay.

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)*.

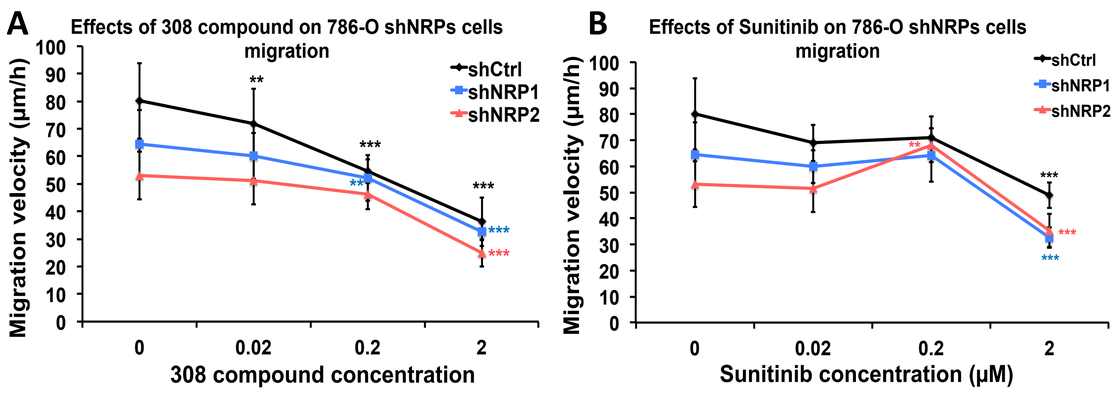


Figure 5. NRP-a308 inhibits 786-O cell migration through NRP-1. Effects of treatments on 786-O cell migration velocity. *A.* Effects of NRP-a308 on the different 786-O shNRPs cells line. *B.* Effects of sunitinib on the different 786-O shNRPs cells line.

Cell Proliferation

The down-regulation of NRP-2 in 786-O cells increases cell proliferation, NRP-2 pathways is anti-proliferative *(figure 2A)*. NRP-a308 decreases cell proliferation in a more efficient way when NRP-1 is expressed on 786-O cells *(figure 2B)*.

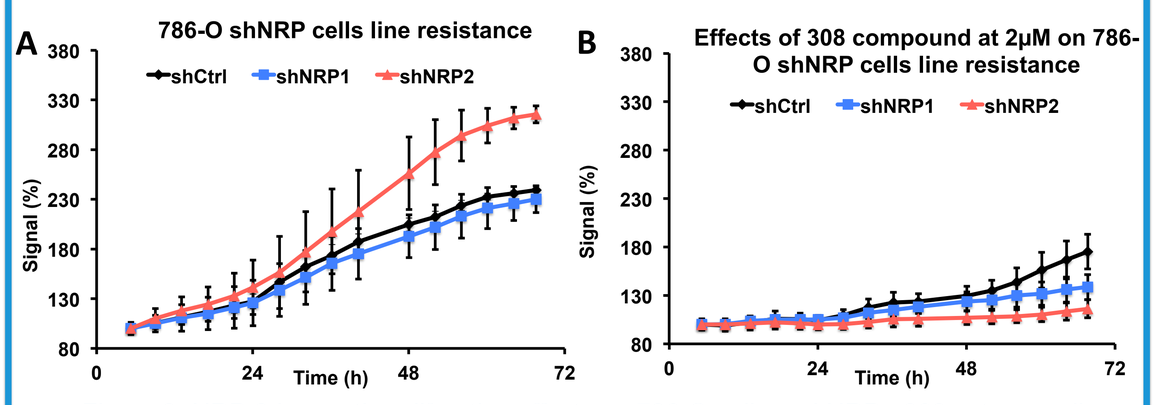


Figure 2. NRP-2 has anti-proliferative effects on 786-O cells and NRP-a308 acts on cell proliferation through NRP-1. A. Effects of NRPs down-regulation on cell proliferation evaluated by cell membrane impedance assays. *B.* Effects of NRP-a308 on cell proliferation evaluated by cell membrane impedance assays.

Cytostatic and cytotoxic effects of NRP-a308

NRP-a308 reduces 786-O shNRPs cell proliferation *(figure 6A)* but has little effects on cell viability *(figure 6B)*. Thus NRP-a308 has a cytostatic effect on 786-O shNRPs cells.

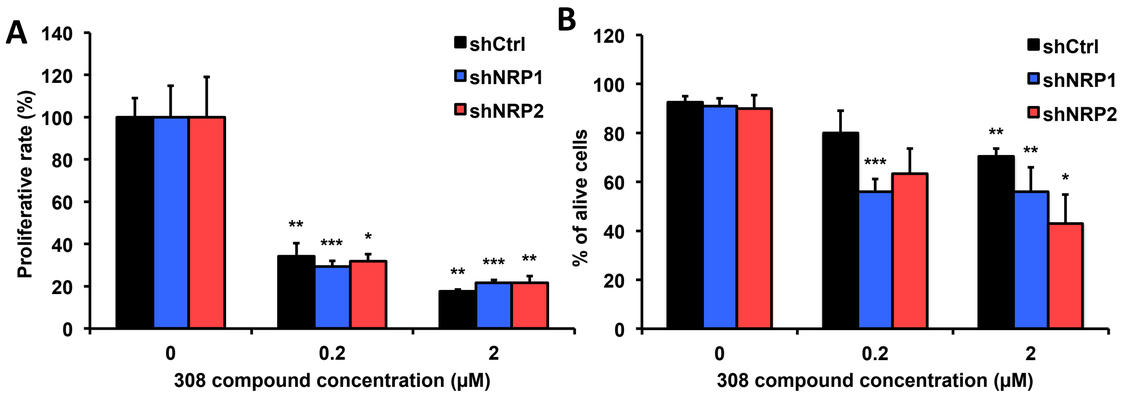
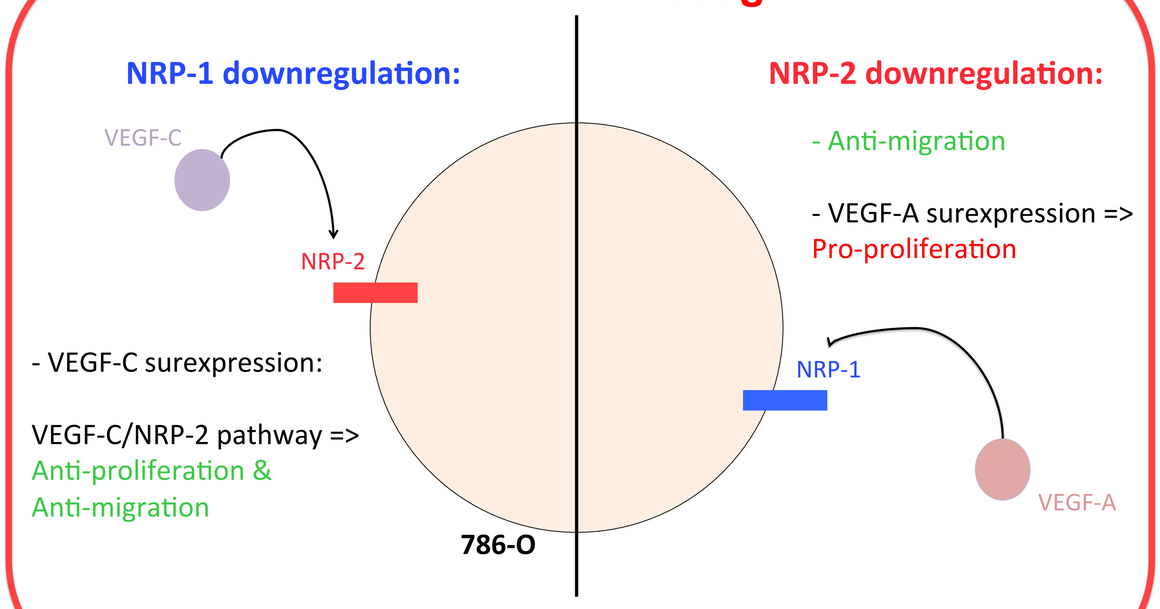


Figure 6. NRP-a308 is cytostatic but not cytotoxic. A. Effects of NRP-a308 on cell proliferation measured by viability assay. *B.* Effects of NRP-a308 on cell viability measured.

Take-Home Message



The NRP-1/VEGF-A rather than NRP-2/VEGF-C pathway should be targeted and inhibited to reduce cell proliferation and migration.

CONCLUSION

NRP-a308 is more efficient than sunitinib, the mccRCC reference treatment on naive mccRCC cells. Its inhibitory effects on proliferation and migration decreased especially when NRP-1 is down-regulated by shRNA. This effect can be explained by the compensatory effect of this two pathways: NRP-1/VEGF-a vs. NRP-2/VEGF-C. Hence, **the NRP-1/VEGF-A pathway should be preferentially targeted**. Therefore, new specific NRP-1 inhibitor are currently developed