S100A4 acts synergistically with VEGF in promoting angiogenesis and a neutralizing monoclonal antibody against S100A4 could be a first in class strategy to combat solid tumors

José Luis Hernández, Sheila Dakhel, Laura Padilla, Jaume Adan, Josep María Martínez, Marc Masa, Ramon Messeguer, Francesc Mitjans, Toni Coll, Rosa Hervas

Biomed Division – Leitat Technological Center. Barcelona, Spain

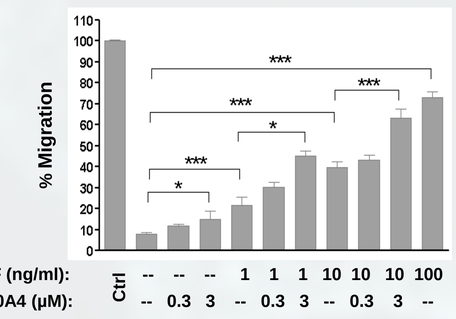
Corresponding author: jlhernandez@leitat.com

Background

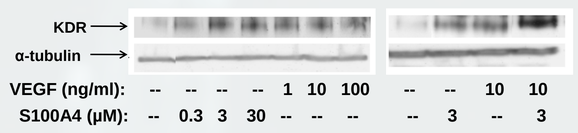
Angiogenesis is one of the most exciting targets for cancer therapy. **Tumor growth** and metastasis are dependent on the ability of the tumor cells to induce and maintain neovascularisation of tumor. Angiogenesis requires an orderly activation of genes controlling proliferation, invasion, migration and survival of endothelial cells. At present, VEGF is the best-validated angiogenic factor and a critical mediator of angiogenesis pathway and several approaches are being used to inhibit VEGF function and treat patients with cancer. Advances in the molecular understanding of the angiogenic cascade and tumorogenesis resulted in the identification of the protein **S100A4**, secreted by tumor and stromal activated normal cells, as a key player involved in endothelial cell migration and therefore a promising factor for **therapeutic** applications interfering tumor angiogenesis and progression.

Results

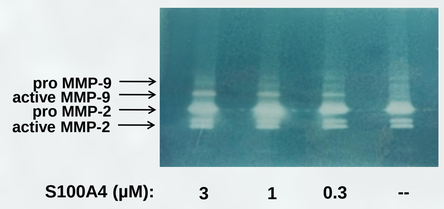
S100A4 acts synergistically with VEGF on endothelial cell migration, increasing KDR protein expression and the production of active forms of MMP-9



Effect of S100A4 and VEGF in HUVECs migration after 24h of stimulation was determined using a transwell system. *Mann Whitney U-test *p<0.05, ***p<0.001*

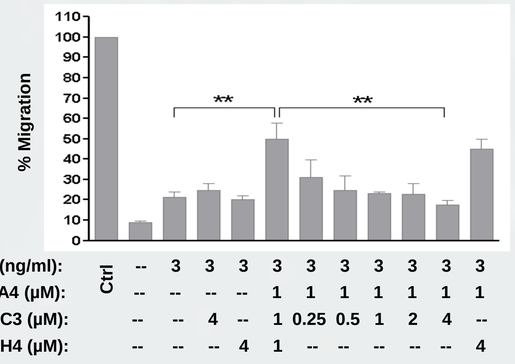


KDR (VEGFR2) expression in HUVECs was analyzed by Western blot after 24h of S100A4 and VEGF stimulation.

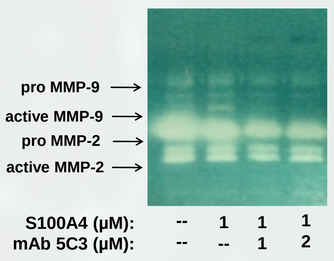


Metalloproteinase activity in HUVECs supernatants after S100A4 stimulation for 24h was analyzed by zymography.

Monoclonal antibody 5C3 against S100A4 have neutralizing activity on induced endothelial cell migration and production of active MMP-9



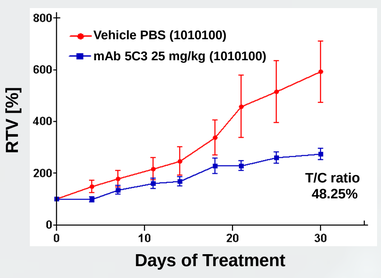
Dose-response neutralizing effect of mAb 5C3 in HUVECs migration induced by S100A4 for 24h. Mab 5H4 was used as non-functional antibody. *Mann Whitney U-test **p<0.01*

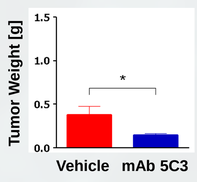


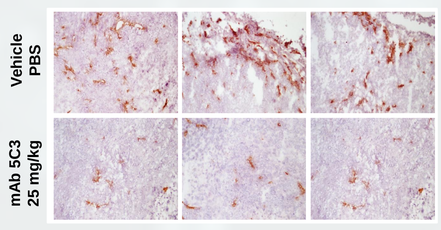
Blocking effect of mAb 5C3 in MMP-9 activity induced by extracellular S100A4 in HUVECs supernatants.

Treatment with mAb 5C3 have inhibitory effect in the growth and vascularization of Mia PACA-2 tumors and potentiates the effect of gemcitabine in vitro

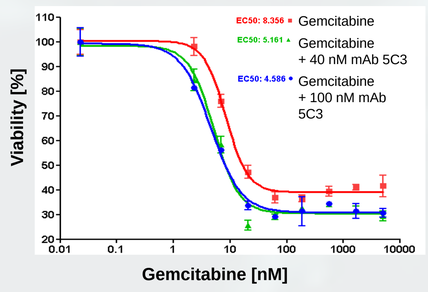
In vivo anti-tumor effect of mAb 5C3. Mia PACA-2 cells (5x10^6 cells/ animal) were inoculated s.c. in the right flank of athymic mice. When tumor volume mean achieved 100 mm^3 mice were randomized in two groups (n=5) and treated with 100 µl i.p. of mAb 5C3 (25mg/kg) or PBS at the indicated dosing schedule. Graph shows mean ± SD. *Mann Whitney U-test *p<0.05*



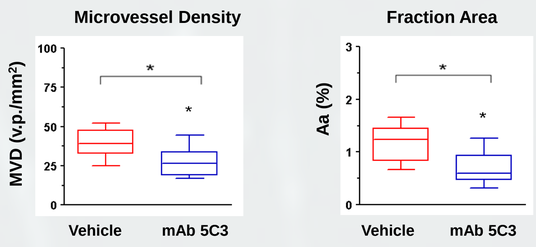




Immunohistochemical staining for CD31 in Mia PACA-2 tumors after 30 days of treatment with mAb 5C3 or vehicle PBS.

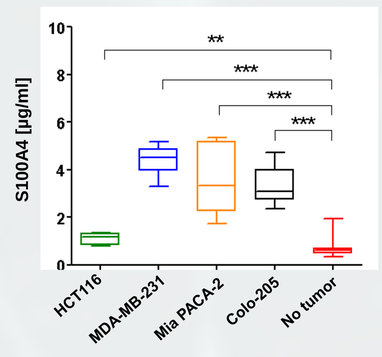


Cell viability analysis using BrdU assay of Mia PACA-2 cells after being treated during 72h with growing concentrations of gemcitabine alone or in combination with mAb 5C3

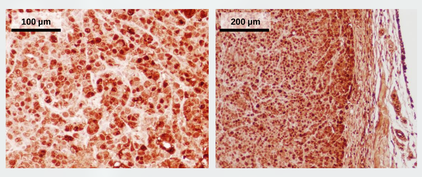


Quantification of density and area fraction of CD31 positive vessels in Mia PACA-2 tumors after 30 days of treatment with mAb 5C3 or PBS. *Mann Whitney U-test *p<0.05*

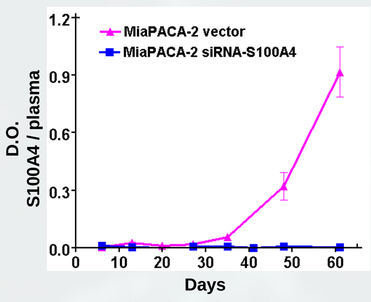
mAb 5C3 is a good diagnostic tool detecting the biomarker S100A4 both in plasma and histological sections



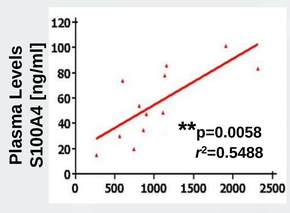
ELISA quantification using mAb 5C3 of S100A4 plasma levels in mice bearing tumors of the indicated tumor cell lines.



S100A4 immunostaining using mAb 5C3 in Colo-205 tumor samples.



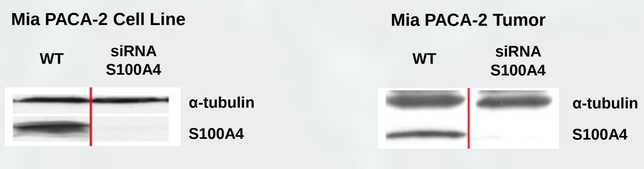
ELISA quantification of S100A4 present in plasma of mice bearing tumors of Mia PACA-2 WT or transfected with shRNA against S100A4



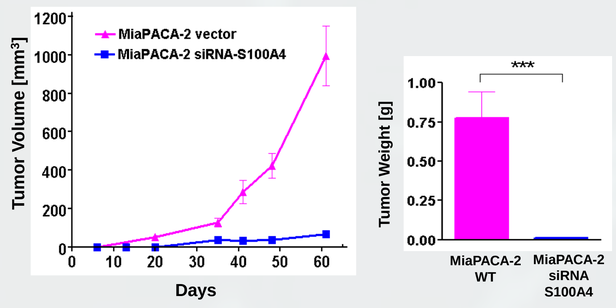
Positive relation between S100A4 plasma level and tumor volume in mice bearing Mia PACA-2 tumor.

S100A4-dowregulation by shRNA in pancreatic cancer cell line Mia PACA-2 reduce dramatically the tumor growth

Western blot analysis of S100A4 expression in wild type and transfected Mia PACA-2 cells *in vitro* and *in vivo*.

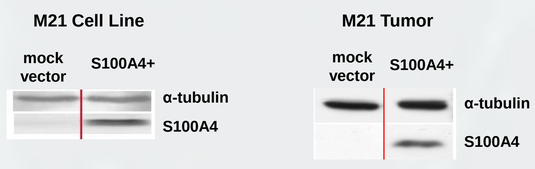


In vivo growth of Mia PACA-2 cells WT and transfected with siRNA against S100A4. Cells (5x10^6 cells/animal) were inoculated s.c. in the right flank of athymic mice (n=5). After 60 days of growth, mice were sacrified and tumor weight and vascularization were evaluated. Graph shows mean ± SD. *Mann Whitney U-test ***p<0.001*

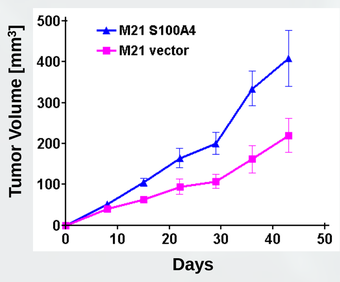


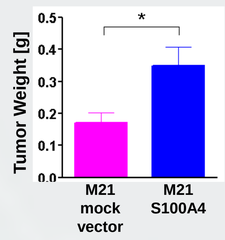
S100A4-overexpressing melanoma cells M21 have a higher growth and a increased vascularization *in vivo*

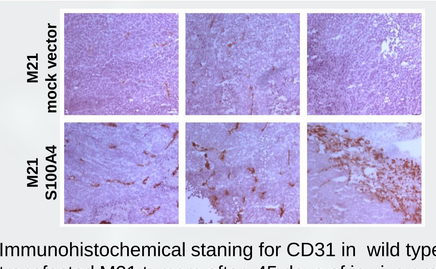
Western blot analysis of S100A4 expression in wild type and transfected M21 cells *in vitro* and *in vivo*.



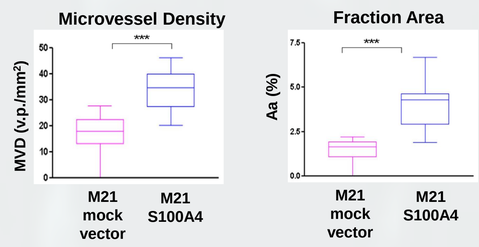
In vivo growing of M21 cells WT and transfected with S100A4. Cells (1x10^6 cells/ animal) were inoculated s.c. in the right flank of athymic mice (n=5). After 60 days of growth, mice were sacrified and tumor weight and vascularization were evaluated. Graph shows mean ± SD. *Mann Whitney U-test *p<0.05*







Immunohistochemical staning for CD31 in wild type and transfected M21 tumors after 45 days of in vivo growing.



Quantification of density and area fraction of CD31 positive vessels in wild type and transfected M21 tumors after 45 days of in vivo growing. *Mann Whitney U-test ***p<0.001*

Conclusion

- S100A4 has a **synergistic effect with VEGF** inducing endothelial cell migration, KDR expression and production of MMP-9, and the monoclonal antibody 5C3 against S100A4 have neutralizing activity on its effect.
- Treatment with **mAb 5C3** have a **inhibitory effect** on Mia PACA-2 tumor **growing and vascularization** and increase the toxicity of gemcitabine *in vitro* when used in combination.
- There is a correlation between **S100A4 plasma levels** and the presence of tumor of several cell lines, and also between plasma levels and tumor volume of Mia PACA-2 tumors. MAb 5C3 is a good **diagnostic tool** to evaluate the presence of S100A4 in body fluids and in tumor biopsies.
- Blockade of S100A4 expression using **siRNA** in Mia PACA-2 **reduce drastically tumor growth** and the **presence of S100A4** in M21 tumor induce an **increase in tumor volume and vascularization** in a xenograph model.

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