New strategies to the inhibition of tumor cell proliferation and tumor metastasis by using S100P monoclonal antibodies

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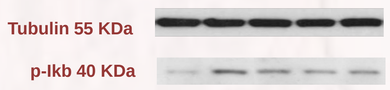
Backgound

Metastasis represents the final, most devastating stage of **malignancy** and the leading cause of death by **cancer**. Cancer cell invasion into the surrounding tissue, systemic dissemination via the vascular system and finally grow of secondary tumors throughout the body are critical events in the multistage process of cancer metastasis. An accurate description of the cellular and molecular mechanisms that underlie this multistep process would greatly facilitate the rational development of therapies that effectively allow metastatic disease to be controlled and treated.

In the last years, intensive research in this field has shed light on some molecular targets as the novel metastatic factor **S100P** protein and its promising role as a key player involved in metastasis formation and poor clinical outcome. The identification and functional characterization of specific regulators of S100P may be exploited for **therapeutic applications**.

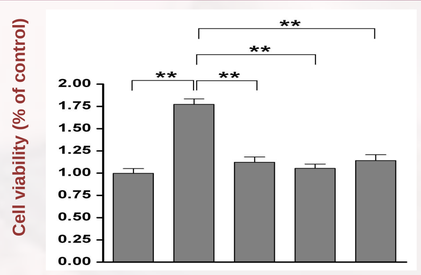
Results

BxPC3



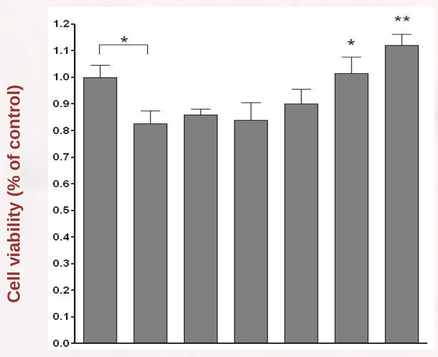
S100P (μM):	Ctrl	1	1	1	1
mAb 2H8 (μM):		--	2	--	--
mAb 1A5 (μM):		--	--	2	--
mAb 3E3 (μM):		--	--	--	2

Ikb phosphorilation was analyzed by Western Blot after 60 minutes with S100P (1μM). The mAbs (2μM) were incubated with the protein 2 hours before addition to the culture.

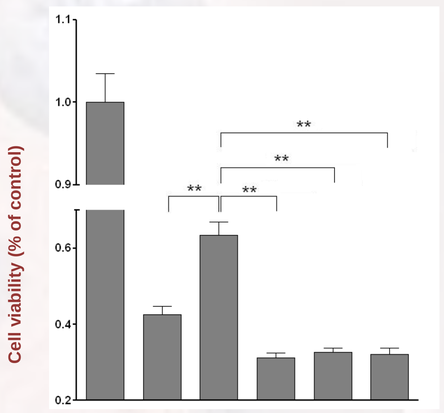


S100P (nM):	Ctrl	100	100	100	100
mAb 3F8 (nM):		--	500	--	--
mAb 1A5 (nM):		--	--	500	--
mAb 2H8 (nM):		--	--	--	500

*BrdU of BxPC3 after 48h of S100P stimulation. The mAb were incubated with the protein 2 hours before addition to the culture. Data shown are the means ± S.E. for nine independent experiments. Mann whitney U-test (**, p<0.01).*



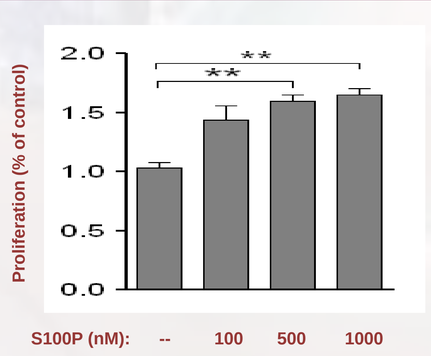
Gencitabine (nM):	Ctrl	23	23	23	23	23	23
S100P (nM):		--	23	47	94	187	750



Gencitabine (nM):	Ctrl	100	100	100	100	100
S100P (nM):		--	500	500	500	500
mAb 1A5 (μM):		--	--	1.2	--	--
mAb 2H8 (μM):		--	--	--	1.2	--
mAb 3E3 (μM):		--	--	--	--	1.2

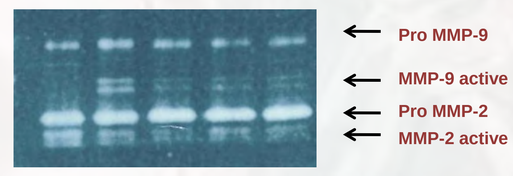
MTT analysis of BxPC3 after 72h of S100P and Gencitabine incubation. mAb were incubated with protein 2 hours before addition to the culture. Data shown are the means ± S.E. Mann whitney U-test (*, p<0.05 / **, p<0.01).

HT1080



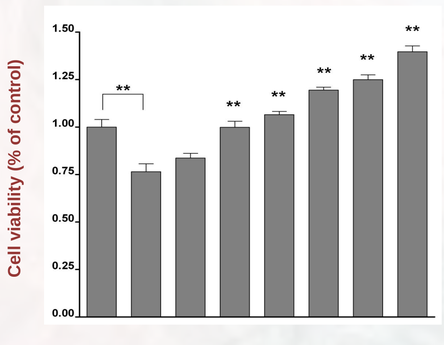
S100P (nM):	--	100	500	1000

*BrdU of HT1080 after 48h of S100P stimulation. Data shown are the means ± S.E. Mann whitney U-test (**, p<0.01).*

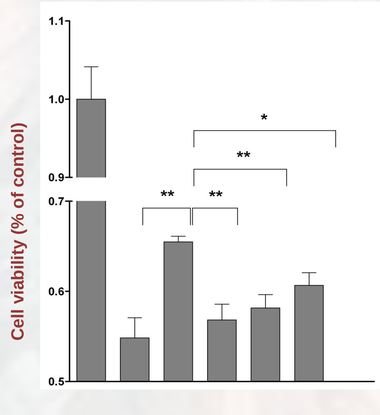


S100P (nM):	Ctrl	100	100	100	100
mAb 3F8 (nM):		--	500	--	--
mAb 1A5 (nM):		--	--	500	--
mAb 2H8 (nM):		--	--	--	500

Gelatin zymography analysis of conditioned medium from HT1080 cell line treated with S100P along 72 hours. The mAb 3F8, 1A5, 2H8 shown an inhibitory effect.



Doxorubicine (nM):	Ctrl	240	240	240	240	240	240	240
S100P (nM):		--	15	31	62	125	250	500

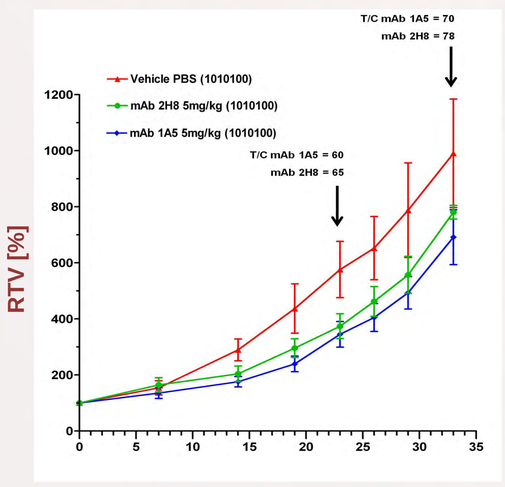


Doxorubicine (nM):	Ctrl	240	240	240	240	240
S100P (nM):			100	100	100	100
mAb 3F8 (nM):		--		500	--	--
mAb 1A5 (nM):		--	--	--	500	--
mAb 2H8 (nM):		--	--	--	--	500

MTT analysis of HT1080 after 72h of S100P and Doxorubicine incubation. mAb were incubated with protein 2 hours before addition to the culture. Data shown are the means ± S.E. for four independent experiments. Mann whitney U-test (*, p<0.05 / **, p<0.01).

mAbs 3E3, 2H8 and 1A5 decrease tumor growth

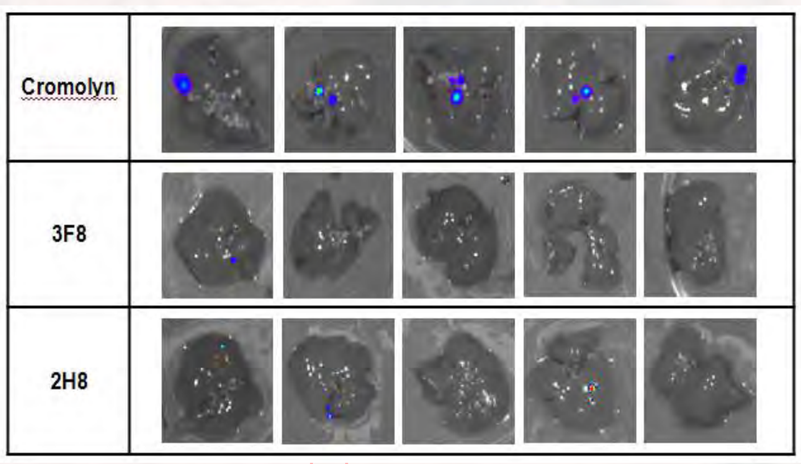
Subcutaneous BxPC3 model



Tumor volume. BxPC3 cells ($4*10^6$ cells/animal) were inoculated s.c. in the right flank of each mouse. Twenty-three days after inoculation, mice were randomly divided into 3 groups (n=5) and treated for 33 days with 20μl Ab 2H8 and 1A5, i.t. at 5mg/kg/3 times per week dosing schedule. PBS was used as vehicle. Tumor volume was measured twice a week. Graph shows mean ± SD. T/C ratio was used as method for efficacy evaluation. Tumor volume was calculated according to the formula $(Dxd^2)/2$.
Efficacy were reflected on the T/C ratio of tumor volume.

mAbs 3F8 and 2H8 confer a less aggressive tumor staging and are good candidates for profiling

Orthotopic BxPC3-Luc model

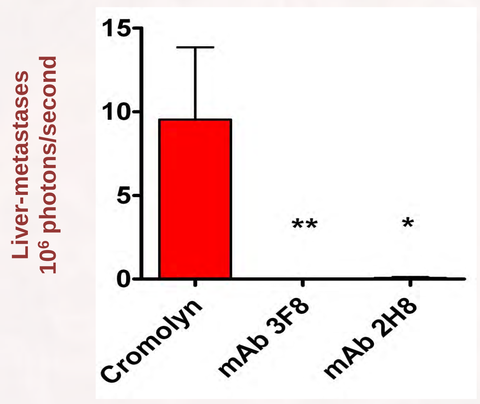


In vivo imaging luciferase represents the liver metastasis in groups treated with cromolyn, mAb 3F8 and 2H8.

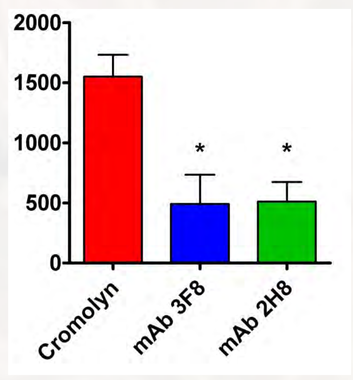
Staging and Scoring System		
Stage	Description	Score
Primary Tumor		
T_0	No tumor	1
T_1	Small tumor (tumor *d<7 mm)*	2
T_2	Large tumor without infiltration (tumor *d>7 mm)*	3
T_3	Large tumor with infiltration but still visible margins	4
T_4	Diffuse and infiltrating tumor	5
Organ Metastases		
M_0	No liver or lung metastases	1
M_{1Li}	Liver metastases	5
M_{1Lu}	Lung metastases	5
M_1	Liver and lung metastases	10
Peritoneal Metastases		
P_0	No peritoneal metastases	1
P_1	Less than five peritoneal metastases or one with *d<7 mm*	3
P_2	More than five peritoneal metastases or one with *d>7 mm*	4
P_3	Malignant ascites	5
P_4	Diaphragm / kidney / intestine / adrenal metastases	$3+3+3+3+P_{0/1/2/3}$
Lymph Node Metastases		
N_0	No lymph node metastases	1
N_1	Peripancreatic lymph node metastases	3
N_2	Regional lymph node metastases (e.g., mesenteric, mediastinal)	5

Scores for the primary tumor (T), organ mestastases (M), peritoneal metastases (P), and lymph node metastases (N) were multiplied to calculate the total tumor score for each animal. Score P_4 value is the sum of the corresponding P_0, P_1, P_2, P_3 ($P_{0/1/2/3}$) plus an additional value of 3 for metastasis presence in the diaphragm, 3 for kidney, 3 for intestine and 3 for adrenal glands.

Quantification and classification system TMPN ("primary Tumor, organ Metastases, Peritoneal metastases, lymph Node metastases").



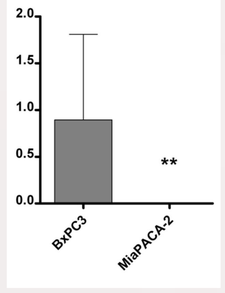
Quantifiable photon emission from in vivo luciferase signaling correlated with liver metastases. Mice implanted orthotopically with BXPC3-Luc in the pancreas. Mice were divided in 3 groups (n= 5) and were treated i.p. for 30 days with cromolyn (5mg/kg), mAb 3F8 and 2H8 (500μg/animal) 3 times per week dosing schedule. PBS was used as vehicle. The luminiscence was mesured two times a week. The graph shows mean ± SD. Mann whitney U-test (, p<0.05 / **, p<0.01)*



Graph shows mean ± SD of the total score for the primary tumor, organ metastases and limph node metastases for cromolyn, mAb 3F8 and mAb 2H8 group. Mann whitney U-test (, p<0.05)*

mAb 1A5, 2H8 and 3F8 are a good diagnostic tools detecting the biomarker S100P in plasma and by IHC

Elisa

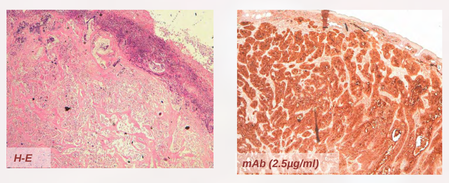


ELISA quantification using mAb of S100P plasma levels in mice bearing tumors of the indicated tumor cell lines. Mann whitney U-test (**, p<0.01)

Western Blot

Western Blot showing the expression of S100P protein in Mia Paca-2 and BxPC3 cells.

Immunochemistry



S100P immunostaining using mAbs in BxPC3 tumor samples.

Conclusion

S100P induces the **proliferation** of BxPC3 and HT1080 cell line and induces the secretion of **MMP-9** in HT1080 cell line and the **mAbs** against S100P have **neutralizing** activity on its effect

S100P promotes the **survival** of BxPC3 and HT1080 cell line that was exposed to Gencitabine and **Doxorubicine** and **mAbs blocked** this activity

MAbs treatment **reduces** pancreatic **tumor growth** and **metastases** in vivo

MAbs 1A5, 2H8 and 3F8 are good **diagnostic** tool to evaluate the presence of S100P in body fluids and in tumor biopsies.

Pub. No.: PCT/ EP2012/050653