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Antitumor activity of bovine pancreatic RNase A *in vitro and in vivo*: the search for molecular targets among miRNAs

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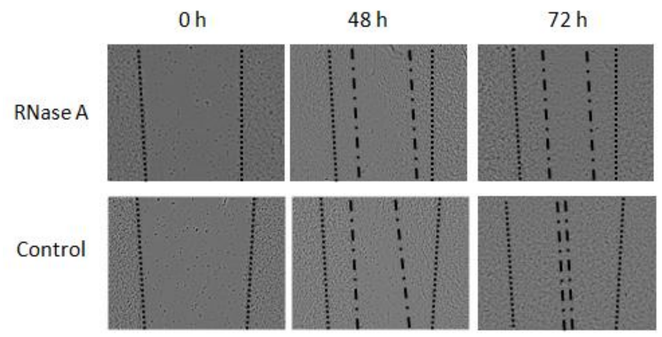
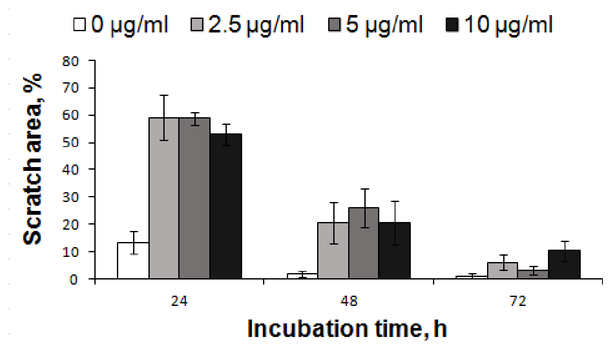
Introduction: miRNAs have been found to be significant modulators of a variety of cellular processes, including angiogenesis , apoptosis , the cell cycle, proliferation and telomerase activity. They are thought to play a key role in disease development, particularly in oncological diseases . An altered miRNA expression profile has been related to the development of different tumours. Exogenous ribonucleases are known to inhibit tumor growth and metastasis via alteration of miRNAs expression profile in tumor cells allowing considering them as promising anticancer drugs for clinical application. In this work the antitumor potential of RNase A was evaluated in vitro and in vivo in Melanoma B16 cells.

In Vitro:



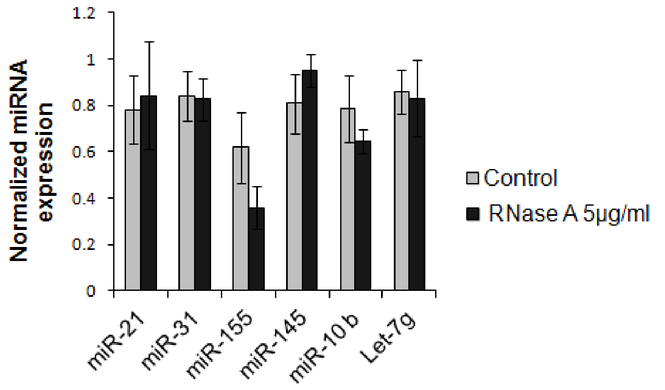
The migration activity of melanoma B16 cells was inhibited by RNase A at 24, 48 and 72 h, compared to control.



B16 cell migration rate in the presence of RNase A

Number in the Library According to RPKM *	Top miRNA (LLC NGSData) (Mironova et al, 2013)	Expression Level of miRNAs##	The Alteration of the miRNA Levels after the RNase A Treatment##
1	mir-21a	0.7	No effect
10	mir-145a	0.8	No effect
15	mir-31	0.75	No effect
29	mir-10b	0.8	1.2 ↓
46	let-7g	0.75	No effect
47	miR-155	0.56	1.7 ↓
U6 snRNA**	0.86		

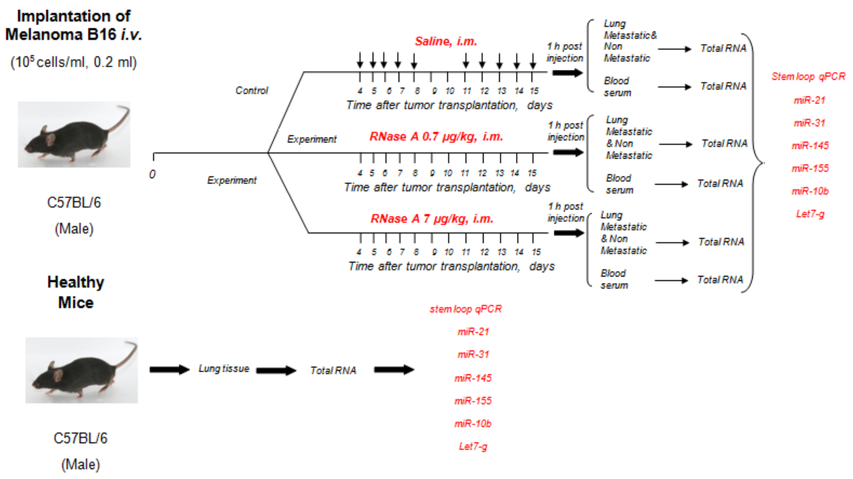
The change in the expression levels of miRNAs under the action of RNase A in melanoma B16.
•RPKM (reads per kilobyte per million): number of reads of specific miRNA/(size of miRNA (kb) _ total number of reads in library (million));
** U6 snRNA was used as an internal control for the miRNA level normalisation; ## miRNA levels normalised toU6 snRNA.



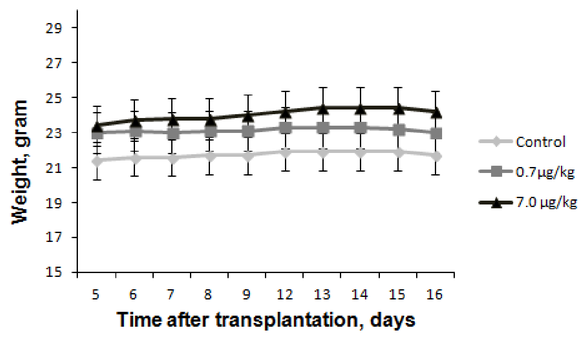
Alteration of the miRNA profile after the treatment of B16 cells with RNase A (5 µg/ml). Data of stem-loop RT-qPCR. miRNA expression levels were normalised to U6 snRNA.

The miRNA profile of melanoma B16 cells was affected by RNase A where the expression levels of oncomir miRNAs (miR-10b and miR-155) were decreased by 1.2- to 1.7-fold.

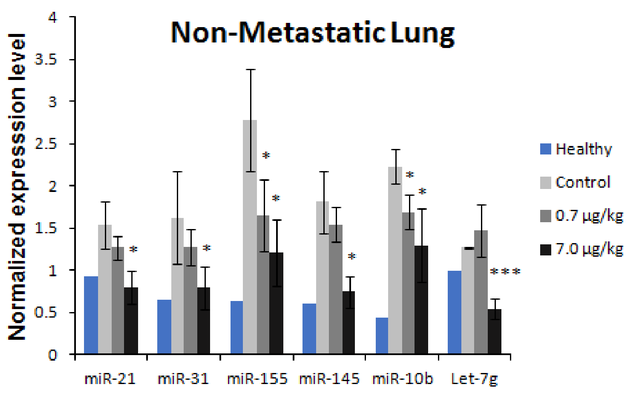
In Vivo:



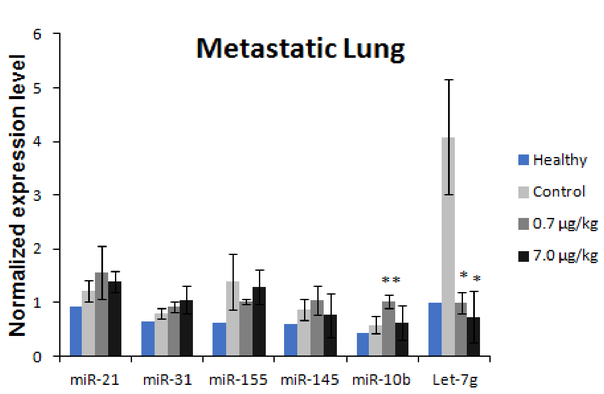
Treatment of B16 by RNase A in vivo. (A) Design of experiment in vivo: B16 cells (10^5 cells, 0.2 ml) were intravenous transplanted into C57BL/6 mice. Starting on day 5 after the tumor transplantation, animals received saline buffer or RNase A intramuscularly daily except weekends for 10 days. Two doses were chosen 0.7 and 7 µg/kg. 1 hour after the last injection, two parts of Lung (metastatic and non-metastatic for comparison and validation and blood samples were collected, total RNA was isolated and miRNA levels were analyzed using Stem loop RT-qPCR .



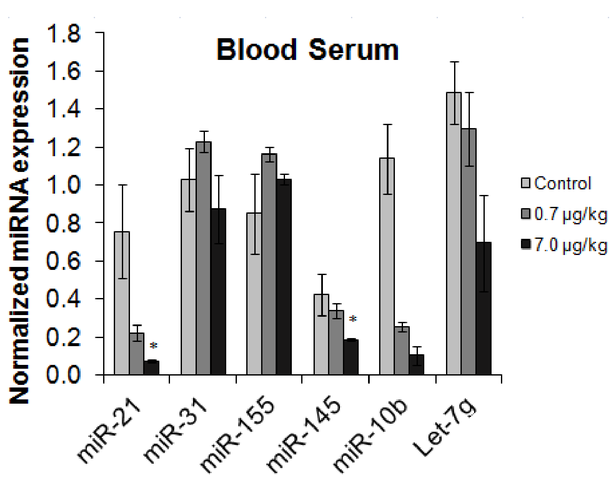
Weight of mice change during the injection period.



In the lung tissue (non-metastatic part) , low (0.7 µg/kg) and high (7.0 µg/kg) doses of RNase A led to a decrease in the expression level of all miRNAs except let-7g, which increased at a low dose, this is due to the combined systemic effects of RNase A in vivo to suppress the progression of metastases.



In the lung tissue (metastatic part), expression levels of oncomir miRNA increased and of tumor suppressor miRNAs decreased. Low dose of RNase A led to an increase in the expression of mir-10b, 145 and 21, while high dose had no effect. Thus, ultralow doses of RNase A (0.7 µg/kg) have the ability to change the process of the biogenesis of miRNAs in the cell B16.



Analysis of the miRNA levels in non-Metastatic lung; the expression level in Metastatic lung and the expression level in the blood serum of mice with B16 after the RNase A treatment at the doses of 0.7 and 7.0 µg/kg. The expression level of miRNAs in the lung tissue was normalised to U6. The concentration of serum-derived miRNAs was normalised to the serum volume.

In the blood serum, levels of four of the six miRNAs analyzed in the serum were decreased, except mir-31 and mir-155, which increased compared to the control group.

Conclusion: Our results suggest that bovine pancreatic RNase A change balance between oncogenic and tumor-suppressor miRNAs brings to the reduction of tumour malignancy resulting in inhibition of metastasis. Bovine pancreatic RNase A can be used as promising antitumor therapeutic and tool for search for miRNA among oncomirs overexpressed upon tumor progression that can be targeted by antisense oligonucleotides or their derivatives.

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