Cancer in voluntary exercising mice at high altitude

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1. Project description

Background Cancer mortality is reduced in human cancer patients living at high altitude (HA). The inverse relationship between HA and cancer mortality was first reported in human leukemia patients back in 1974. In the last decades these findings were confirmed after correcting for confounders such as industrialization, urbanization and ethnicity by several independent studies [1-4]. A recent study compared cancer mortality for Caucasians living at high and low elevated counties in the US [5] and showed a clear correlation between HA and reduced overall cancer mortality. Although overall cancer mortality negatively correlates with HA [2], cancer is a heterogeneous group of diseases that of course share many features but that also differ in etiology and pathology. Thus, site-specific cancer types may respond differently to HA: Indeed, HA exposure reduces mortality in lymphoma, breast [6], lung, tongue and mouth or larynx cancer [7, 8] but increases mortality in melanoma due to the higher background radiation at HA [9].

A lower malignancy at HA seems surprising because tumor tissue hypoxia *per se* promotes tumor growth and the development of aggressive phenotypes [10, 11]. Aggressive phenotypes develop even faster in combination with tumor-associated or therapy-induced anemia. However, in contrast to acute disease-associated anemia, humans living at HA prior to tumor onset are systemically adapted to hypoxia. HA-adapted highlanders reach a physiological and cell biological steady state that differs from the lowlander steady state. This difference might be the reason for reduced cancer mortality at HA and might hold a key to improve cancer treatment. Besides exposure to hypobaric hypoxia, highlanders may also have a different live style than lowlanders: Highlanders might be more physically active than lowlanders because the infrastructure as well as the job profiles at HA (e.g. agriculture). High physical activity improves all stages of cancer disease from incidence to proliferation to metastasis formation and to treatment success. Thus, we used a mouse model of lung metastasis to study how a combinatory stimulus of HA exposure at the JFJ and physical exercise (voluntary running in running wheels) impacts on metastasis formation.

Results in 2019 – Metastasis in physically exercising mice at HA
Tumor malignancy and metastasis formation are the major reasons for cancer-related mortality. Lung invasion and colonization is an important part of metastasis formation. We used C75BL/6 male mice and intravenously injected B16F10 melanoma cells, which invade lung tissue, as a metastatic mouse model. The B16F10 melanoma cells were bioengineered to express the green fluorescent protein (GFP) for identifying cancer cells within the mouse tissue. We used histological methods (Immunohistochemistry for GFP as well as Hematoxylin and Eosin staining) to quantify metastasis in tissue section. We also manually counted cancer colonies because melanoma cells express melanin and thus, colonies appear black on the lung surface. We used real time PCR to estimate metastasis in lymph and blood circulation by analyzing the mRNA expression of the GFP marker.

Our results show that B16F10 melanoma cells invaded the lung after i.v. injection (Fig.1 A). HA exposure reduced the number of metastatic colonies in both, running and non-running mice (Fig.1 B) suggesting that HA exposure *per se* may protect from lung invasion. Surprisingly, voluntary running increased the number of metastasis at low altitude (but not at high altitude) (Fig.1 B). This suggests that physical exercise may not always be beneficial but rather requires an optimized trainings program (in our mouse model but potentially also in human patients). Histological analyzes showed similar results (not shown). The analysis of cancer cells that circulate in lymph and blood showed that neither HA exposure nor physical exercise influenced the number of circulating cancer cells (not shown). We also analyzed and scored the infiltration of lung metastasis by immune cells. A score of 0 and 1 represent no or a low-grade inflammation and a score of 2 and 3 a high grade inflammation (detailed description of scoring in [12]). We observed that both HA exposure and physical exercise decreased the inflammatory response with exercising at HA displaying the lowest tumor inflammation (Table 1).

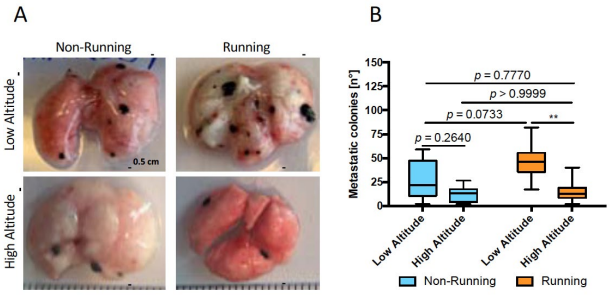

*Figure 1. Quantification of melanoma metastases in running and non-running C57BL/6 mice either at low or at high altitude. Shown is (A) a representative images of lungs (ventral side) with B16F10 melanoma metastasis. Top left, Low altitude Non-Running; Top right, Lo Altitude Running; Bottom left, High Altitude Non-Running; Bottom right, High Altitude Running. (B) Quantification of metastatic colonies on lungs of non-running (blue) and running (orange) mice either exposed to low or to high altitude. Colonies were manually counted, and results are shown as boxplot with median and whiskers of min and max. Scale = 0.5 cm. Statistical analysis: 2-way ANOVA with Bonferonni post hoc analysis for multiple comparison (n = 11 for non-running groups and n = 8 for running groups), **p < 0.01.*

Table 1. Distribution of immune cell score in lung B16F10 metastasis

Group	Score				Mean score	Confidence interval (95%)	N
	0	1	2	3			
LANR	2	9	0	0	0.82	0.57-1.07	*11*
HANR	6	6	0	0	0.55	0.24-0.86	*11*
LAR	1	2	5	0	1.50	0.98-2.02	*8*
HAR	6	2	0	0	0.25	0.00-0.57	*8*

All data unpublished, taken from Master thesis Nina Desboeufs

In conclusion, our data show that HA exposure but not physical exercise may reduce the number of lung metastasis in our mouse model of tail-vein injected B16F10 melanoma cells. However, both stimuli individually and together reduced the recruitment of immune cells to the lung metastases. We will test if we observe similar effects in a second mouse model to estimate if HA or exercise or both interfere with lung metastasis and thus reduce cancer mortality.

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Internet data bases

http://www.vetphys.uzh.ch/index.html

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Refereed journal articles and their internet access

Thiersch, M., and E.R. Swenson, Cancer at high altitude, High Altitude Medicine & Biology, **19**, 2, 116-123, doi: 10.1089/ham.2017.0061, 2018. https://doi.org/10.1089/ham.2017.0061

Conference Papers

Simpson, F., N. Desboeufs, A. Leimbacher, T. Le, G. Cosi, T. Haider, M. Gassmann, M. Thiersch, Effect of high altitude exposure and voluntary exercise on cancer growth and metastasis, 15th Symposium of Zurich Center for Integrative Human Physiology (ZHIP), Zurich, Switzerland, August 23, 2019.

Simpson, F., N. Desboeufs, A. Leimbacher, T. Le, G. Cosi, T. Haider, M. Gassmann, M. Thiersch, Effect of voluntary exercise on cancer growth and metastasis at high altitude, Poster presentation at the 2nd Poster and Networking day, Vetsuisse Faculty, Zurich, Switzerland, September 25, 2019.

Theses

Desboeufs, N., The impact of high altitude exposure and physical exercise on metastasis formation and spreading, Master Thesis, University of Zurich, Start 2019, End February 4th 2020.

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