Reduced metastasis in high altitude exposed mice

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1. Project description

Background

Living at high altitude (HA) lowers cancer mortality over a broad spectrum of cancer types in highlanders in North America and Europe (reviewed in (Thiersch, Swenson et al. 2017, Thiersch and Swenson 2018)), which suggest that common tumor suppressive mechanisms are activated at HA. Physiological acclimatization and adaptation at HA are predominantly driven by reduced oxygen uptake (hypoxia). Chronic exposure to HA results in adaptation of healthy cells and organs to lower oxygen levels ahead of tumor formation. It may be possible that adapted, healthy cells reach a steady state allowing them to compete with tumor cells for space and nutrition in their niche (e.g., with tissue-invading circulating cancer cells). Understanding the mechanism that lower or prevent competitiveness of cancer cells (and thus tumorigenesis) may help developing novel, oxygen-based therapeutics.

Like humans, rodents (including mice) showed a lower cancer mortality and reduced metastasis at HA (reviewed in (Thiersch, Swenson et al. 2017, Thiersch and Swenson 2018)) during acute exposure (Yu and Hales 2011) as well as after acclimatization to 10% oxygen levels at low altitude (LA) (isobar hypoxia) (Sung, Ma et al. 2011). Thus, mice are adequate models to analyze the impact of HA on cancer. In our previous experiments, we observed that HA exposure did not alter the growth rates of primary tumors but prevented or reduced severity of anemia of cancer (anemia = impaired red blood cell RBC production resulting on reduced oxygen transport capacity) as well as reduced B16F10 melanoma lung metastasis. Metastasis is the main reason for cancer-based death and metastatic cells typically originate from tumor regions that are poorly supplied with oxygen (tumor hypoxia). To evaluate if the effect of HA on metastasis can be observed in second lung metastasis mouse model, we established a LLC1 lung cancer metastasis model by injection LLC1 lung cancer cells intravenously in LA and HA exposed mice. Additionally, we analyze if physical exercise (running in running wheels) supports the effect of HA. Although our previous experiments already suggested that exercise had no big effect in our cancer models (see report 2019), we verified these findings in a second model.

Results in 2021 – Reduced metastasis mice at HA

We used C75BL/6 male mice and intravenously injected LLC1 lung cancer cells, which invade lung tissue, as a metastatic / lung cancer mouse model. We used histological methods (Immuno-histochemistry, as well as Hematoxylin and Eosin staining) to quantify metastasis in tissue section and manually counted cancer colonies.

Our results show that LLC1 melanoma cells invaded the lung after i.v. injection (Fig. 1 A) and HA exposure reduced the number of metastatic colonies (Fig. 1 B) and prolonged the survival of mice with lung metastasis (Fig. 1 C) confirming that HA exposure *per se* protects the lung from invasion of circulating cancer cells. As expected, physical exercise had no effect on metastasis. Histological analyzes showed that immune cells (macrophages, T-cells, NK-cells were recruited to growing cancer colonies in the lung. However, we observed no difference (by immunohistochemistry) in number and localization of immune cells in metastases of LA and HA exposed mice. Our currently investigated hypothesis is that circulating cancer cells are either less invasive at HA or the lung (as a host tissue) of HA exposed mice is more protected from invading cancer cells. To test if elevated hematocrit (i.e. increased blood viscosity and therefore higher shear forces) lowers the invading potential of circulating cancer cells, we injected C57Bl/6 mice at LA with EPO or saline to match the hematocrit (ca. 50-60%) of HA exposed mice or not. When we intravenously injected LLC1 cancer cells into these mice, both saline and EPO treated mice developed similar number of metastases, suggesting that blood viscosity had no impact on circulating cancer cells in our model. Thus, we will investigate in the future if the lung of HA exposed mice is better protected from invading cancer cells.

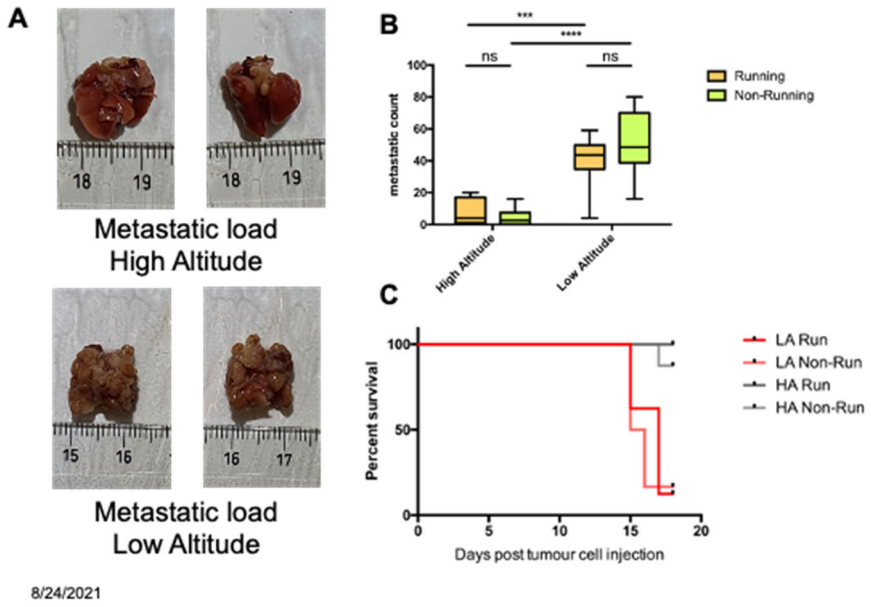

*Figure 1. Analysis of LLC1 metastases in exercising (running) and non-exercising (non-running) C57BL/6 mice either at low (LA) or at high altitude (HA). Shown is (A) a representative image of lungs with LLC1 lung metastasis. Scale = 0.5 cm. (B) Manually counted lung cancer colonies of exercising (running, orange) and non-exercising (non-running, green) mice acclimatized to either low LA or high altitude (HA) are shown as boxplot with median and whiskers of min and max. Scale = 0.5 cm. Statistical analysis: 2-way ANOVA with Bonferroni post hoc analysis for multiple comparison (n = 11 for non-running groups and n = 8 for running groups), ***p < 0.001. (C) Survival of mice with LLC1 lung metastases lungs of non-running (Non-Run) and running (Run) mice either exposed to low (red) or to high altitude (grey).*

All data unpublished, taken from Master thesis Philipp Villiger.

In conclusion, our data show that HA exposure may reduce the number of lung metastasis, while we confirm that physical exercise has no major effect on metastasis. After showing that increased hematocrit did not alter the invading potential of circulation cancer cells, we will now test if the lung of HA acclimatized mice is better protected from invading cancer cells.

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Internet data bases

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Manuscript on exercise and cancer as well as on high altitude and cancer are planned for 2022

Theses

Philipp Villiger, Title TBA. Master Thesis, University Zurich, Start 2021, End April 31st, 2022

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