The role of myoglobin in the tumorigenesis of breast cancer: *in vitro* and *in vivo* models for analysis of tumor incidence, proliferation, virulence and response to radiotherapy

Project description: Mammalian myoglobin (MB) is a cytoplasmic heme-containing respiratory protein of cardiac myocytes and oxidative type I/IIa skeletal muscle fibers. In striated human muscles MB occurs at concentrations of ~300–500 μM. At this abundance, the monomeric globin is widely accepted to function as a temporary "store" for oxygen, able to buffer short phases of exercise-induced increases in O_2 flux during which it supplies the gas to mitochondria. However, our recent immunohistochemical (IHC) survey of 1000+ breast cancer specimens revealed a surprisingly widespread expression of MB protein in human breast carcinoma and normal breast parenchyma biopsies. Roughly ¾ of breast tumors displayed MB protein expression in significant correlation with a positive hormone receptor status and better prognosis, suggesting MB positivity as a novel diagnostic marker for the recognition of the well-differentiated, less aggressive luminal breast cancer subtype. Healthy and cancerous breast tissue from "mice and men" express MB specifically within the secretory luminal cells of the 2-layer comprising milk-duct epithelium, where the oxygenated protein (MBO_2) might act as a putative shuttle for fatty acids in support of active lipogenesis, mitochondrial β-oxidation and, ultimately, cellular growth at times of non-limiting O_2 supplies. In cultured breast cancer cells, MB expression occurs *de novo* and in hypoxia-inducible, HIF-participated fashion. However, MB protein levels in these cells are far below (~several hundred-fold) those found in myocytes of striated muscles. Thus, one of central questions of this work addresses the link between amount and function of a protein. In other words: is MB, at nano- or low micromolar concentration, still able to effectively oxygenate breast epithelia as it is in muscles, or does the protein take on new activities that are not directly related to O_2-transport?

Breast cancer is the most common cancer of women, affecting 1 in 6 women. It is the second leading cause of cancer-related death among woman. In times of debated mammographic mass screening and surgical intervention of even small and indolent breast malignancies (e.g. DCIS), the discovery of reliable markers permitting stratification of tumor subtype, grade and treatment strategy are of utmost importance. The fact that MB expression in breast cancer cells reflects a less virulent tumor entity, and thus marks a good candidate to serve as additional outcome predictor in the treatment of breast cancer patients, has also inspired us to examine the mechanisms by which MB impacts tumor cell survival and behavior. Initial experiments indeed illustrated that endogenous, breast cancer cell-expressed MB is able to promote proliferation (see above) and apoptosis but, paradoxically, that it also impairs the mitochondrial activity of these cells. Moreover, increasing evidence points to a genetic interaction between MB and the tumor-suppressor p53, whose dysregulation underlies the development of every second human mammary carcinoma. Based on our preliminary data we, thus, hypothesize that MB should increase the efficacy of chemo- or radiotherapy (see above: oxygenation-dependent?), and that it governs breast tumorigenesis in p53-independent and p53-dependent ways.

To test our hypotheses, we have, thanks to recent funding (below), started to generate novel cell and animal models including CRISPR/Cas-mediated MB and p53 knock out (MBko and p53ko) breast cancer cell clones and mouse models with spontaneous development of mammary tumors (a) PyMT; b) WapCre;$p53^{flox}$) in a MB-proficient (MBwt) and -deficient (MBko) background. We are also planning to isolate breast cancer stem cells (BCSC) from these tumor-bearing mice to analyze MB`s role of in early tumorigenesis along with its expression pattern throughout different stages of tumor development in correlation with spontaneous p53 mutations. Overall, these new experiments aim to explore i) the biological role of MB and especially its interaction with p53 in breast cancer and breast cancer stem cells for assessing the globin as candidate tumor suppressor; ii) MB´s expression along the different stages of tumor development (from BCSCs to overt malignancies) for further evaluation of MB´s suitability as diagnostic marker, and iii) MB´s potential interference with certain therapeutics, perhaps even at the far lesser concentrations of breast cancer cells (above). Unraveling MB´s novel roles in restricting tumor virulence will hopefully also provide innovative strategies for coming breast cancer interventions.

Key words:
Myoglobin, Globin, Breast Cancer, Hypoxia, HIF, ERα, FASN, Estrogen Signaling, Fatty Acids, Lipogenesis

Involved Personal:
Project is made possible through a 4-lab collaboration. In addition to our own group, major contributions to the project have been provided by 3 other groups that are represented by the PI`s name under "Collaborations".

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Funding:
i) Swiss Cancer Research (Krebsforschung Schweiz): March 1st, 2016: total of 242'450 CHF for 3 years; ii) Marie-Louise von Muralt Stiftung für Kleintiere: April 1st, 2016: total of 140'000 CHF for 2 years; 3rd year extension possible.

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Duration:
March 2015 – Feb 2018