

**PhD Thesis Title:** Study of the destructive effects of shear stress and ROS-generating drugs on circulating tumor cells

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## **ABSTRACT:**

Cancer metastasis is a complicated disease that causes around 90% of cancer deaths. It is one of the deadly diseases around the world killing almost 8.8 million people in 2015. Exercise, anti-cancer drugs, and antioxidants have attracted the interest in the rehabilitation of the cancer patients. Insights on the mechanism of prevention facilitate the treatment strategy of the disease. Hence, this thesis mainly focuses on the shear stress (SS)-based strategy for studying the metastatic properties of circulating tumor cells (CTCs) in a microfluidic system in the view of exercise, anti-cancer drugs, and antioxidants, to develop therapeutic strategies to prevent the cancer progression. CTCs are the primary targets of cancer treatment as they cause distal metastasis. However, how CTCs respond to exercise-induced high SS is largely unknown. To study the effects of hemodynamic microenvironment on CTCs, we designed a microfluidic circulatory system that produces exercise relevant SS. We explore the effects of SS on breast cancer cells with different metastatic abilities, cancer cells of the ovarian, lung and leukemic origin. High SS of 60 dynes/cm<sup>2</sup> is achievable during intensive exercise killed more CTCs than low SS of 15 dynes/cm<sup>2</sup> present in human arteries at the resting state. High SS caused necrosis in over 90% of CTCs within the first 4 hours of circulation. More importantly, the CTCs that survived the first 4 h-circulation, underwent apoptosis during 16-24 h of post-circulation incubation. Prolonged high SS treatment effectively reduced the viability of highly metastatic and drug-resistant breast cancer cells. As high SS had much less damaging effects on leukemic cells mimicking the white blood cells, we propose that intensive exercise may be a good strategy for generating high SS that can destroy CTCs. To increase the relevance of the previous study and to develop a potential platform for the discovery of anti-cancer drugs with the intention of killing CTCs, we further tested the drug model in this microfluidic system. Many anti-cancer drugs are in practice in the clinic; however, little is known about their efficacy in CTCs. In this thesis, we investigated whether the pulsatile fluidic SS in human arteries can affect the efficacy of anti-cancer drugs. Cancer cells were circulated in our microfluidic circulatory system, and their responses to drug and SS treatments was observed. The results showed that fluidic SS significantly increased the potency of the reactive oxygen species (ROS)-generating drugs doxorubicin (DOX) and cisplatin but had an insignificant effect on the non-ROS-generating drugs Taxol and etoposide. Co-treatment with SS and ROS-generating drugs dramatically elevated ROS levels in CTCs, while adding antioxidants abolished the apoptotic effects of DOX and cisplatin. More importantly, the synergistic killing effects of SS and DOX or cisplatin were confirmed in the circulated lung, breast, and cervical cancer cells, some of which have a strong metastatic ability. These new findings suggest that ROS-generating drugs are more potent than non-ROS-generating drugs for destroying CTCs under pulsatile fluidic conditions. This latest information is highly valuable for developing novel therapies to abolish CTCs in the circulation and prevent metastasis. Finally, the survival capabilities of lung metastasis-derived cell lines that were isolated from mice models in our lab were also studied in this thesis. The metastasis-derived breast cancer cells experienced increased basal levels of survival and underwent stronger resistance even during the high shear stress induced by exercise conditions for 4 h compared to the parental breast cancer cell line. However, the metastasis-derived cells could not resist the high shear stress for 8 h. This proves the fact that a high SS can also destroy highly metastatic and drug-resistant breast cancer cells. Furthermore, metastasis-derived cells revealed differences in their survival

capabilities. This contributes to increasing evidence of enhanced survival capabilities in cancer progression, metastasis and provides an insight into the role of killing the CTCs. As high SS had much less damaging effects on leukemic cells mimicking the white blood cells, we propose that intensive exercise may be a good strategy for generating high SS and use of ROS generating drugs that can destroy CTCs and prevent cancer metastasis. Overall, I studied three important aspects in cancer metastasis: SS based exercise strategy, anti-cancer properties of drugs and the role of antioxidants during the circulation in a microfluidic system. I have revealed new insights correlating the exercise-induced SS to the survival of CTCs and provided the evidence of survival capabilities linked to reactive oxygen species (ROS) inducing drugs and non-reactive oxygen species (NROS) inducing drugs mechanism in cancer metastasis. Understanding how the fluidic SS with anti-cancer drugs influences the survival of tumor cells provides new insight into the cancer metastasis and have important application in the treatment of cancer. Discovering these new insights in the areas of cancer prevention may be an effective therapeutic target in the treatment of cancer.

#### References to author publications that relate specifically to the dissertation:

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3. **Sagar Regmi**, Chetan Poudel, Rameshwar Adhikari, and Kathy Qian Luo. "Applications of microfluidics and organ-on-a-chip in cancer research." *Biosensors* 12, no. 7 (2022): 459. <https://www.mdpi.com/2079-6374/12/7/459>
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