# The role of vitamin D receptor in 5-fluorouracil responsiveness of colorectal cancer cells

Despite the development of surgical skills and the introduction of novel medicines to the clinical setting in the last two decades, colorectal cancer (CRC) is still one of the leading causes of cancer-related deaths worldwide. Unfortunately, drug-resistance is one of the main reasons for the low survival rates of CRC patients. 5-Fluorouracil (5-FU) is the most commonly used drug in the clinical treatment of CRC today and remains a backbone of systemic therapy for patients with locally advanced and metastatic CRC. However, the clinical benefits of 5-FU treatment are often temporary, and the majority of treated patients do not achieve complete eradication of tumor cells, resulting in poor outcomes due to recurrence after 5-FU therapy. The effectiveness of treatment is seriously hampered since patients are prone to develop resistance to 5-FU. *Given the above, the issue of how to improve the efficacy of 5-FU therapy and to reverse or to prevent 5-FU resistance are still relevant challenges in clinical practice. Accordingly, a better understanding of the mechanisms which regulate the acquisition of resistance to 5-FU-based therapy would be a high benefit for the development of more effective regimens for CRC patients' treatment.*

The long-term cell exposure to 5-FU results in an acute increase in the expression of thymidylate synthase (TS) protein because of the enhanced translational efficacy of *TYMS* mRNA. In turn, among resistance mechanisms reported so far, the high TS expression represents a crucial factor in 5-FU resistance. *In our studies, we discovered that CRC cells with silenced vitamin D receptor (VDR) after exposition to 5-FU significantly increased TYMS expression. Our findings identify a previously unrecognized role of unliganded VDR in TYMS expression after CRC cell exposure to 5-FU, thus its significance in the cell resistance to 5-FU.* Therefore, the project aims to determine whether the level of VDR in CRC cells could influence the effectiveness of 5-FU anticancer activity. We hypothesize that VDR plays a significant role in the regulation of the degree of CRC cell responsiveness to 5-FU. We suppose that reduced or lost expression of VDR is a critical contributor to the development of CRC cell resistance to 5-FU, probably mainly via increasing FOXM1-mediated expression of *TYMS* and consequently enhances CRC cell stemness, invasion, and migration. While the high VDR level in CRC cells could give the opposite effect and lead to an increase in the efficacy of 5-FU anticancer activity. Additionally, we expect that unliganded VDR could also blockade the Hsp90-Src pathway preventing its activation and also causing the decrease of *TYMS* expression in CRC cells. Whereas increased expression and activity of Hsp90 following 5-FU treatment could enhance CRC cell sensitivity to vitamin D derivatives (VDDs). The level of VDR is closely related to the initiation and development of CRC. The expression of VDR increases in precancerous lesions and early stages of colorectal tumorigenesis, but decreases in late-stage poorly differentiated tumors and is absent in associated metastases. Therefore, the elucidation of whether VDR level in CRC cells influences the effectiveness of 5-FU anticancer activity is extremely important.

In our research, we plan to explain whether VDR loss accelerates and the high VDR level delays the development of CRC cell resistance to 5-FU. We will investigate both whether VDR regulates TYMS expression directly via FOXM1 and whether VDR interacts with Hsp90α/β and thus its influence on Hsp90-Src pathway activity. Next, we will evaluate the responsiveness of 5-FU-resistant CRC cells to VDD and 5-FU and the role of Hsp90β in VDDs activity against these cells. Furthermore, we will elucidate whether VDDs play a role as OXPHOS inhibitors against 5-FU-resistant CRC cells following treatment with 5-FU.

The understanding of the mechanisms underlying the occurrence of 5-FU resistance is an essential step toward increasing the effectiveness of 5-FU-based therapy and thus delaying recurrence of disease and extending the survival of patients. The proposed research will elucidate the mechanisms underlying the VDR-mediated TS regulation and answer the question of whether VDR level in CRC cells affects the efficacy of 5-FU anticancer activity. We suggest that VDR could be a novel predictive biomarker in 5-FU-based therapy for CRC patients. In this context, this project will contribute to the development of basic science and expand the knowledge in the field of oncology as well as provide a new theoretical basis for the establishment of more effective strategies in the treatment of CRC patients.