Targeting SUMO signaling to wrestle cancer

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Cover

A splash of water color paint, every droplet fighting to be the biggest of all. A representation of a drug and a cancer cell fighting to be the last one standing. With work done in this thesis and research all over the world the hope is that splash of "drug" will overpower the darkness of the cancer. We all know that this is not reality yet as we remember the people we lost. The task is to continue the work to generate more colorful splash.

Colophon

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Geniet en kies avontuur, want ooit is zo ver weg!

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THESIS OUTLINE

SUMOylation plays a widespread role in carcinogenesis; multiple players of the enzymatic SUMO cascade are up or downregulated in several cancer types. The role of SUMOylation in carcinogenesis, consequently led to the interest in targeting of SUMOylation for cancer therapy. In recent years drugs targeting SUMOylation have been developed, specifically small molecule inhibitors targeting the E1 enzyme of the SUMOylation cascade show potential. This thesis focusses on the effectiveness of these inhibitors and their therapeutic potential, via identification of cancer dependency on SUMOylation, investigating the cytostatic role of SUMO in cell cycle and the repressive role of SUMO in immunity and identification of combination therapeutic strategies.

A review on the role of SUMO in cancer and how to target SUMO signaling is presented in **Chapter 1**. We discuss SUMOylation and its roles in cell cycle progression via multiple cellular mechanisms highlighting the potential of SUMOylation inhibitors to block cancer cell division. Next, we highlight the role of SUMO in the regulation of tumor suppressor p53 and oncogene c-Myc. In addition, we discuss the role SUMO plays as a master repressor of gene expression in immune signaling cascades, marking the potential for SUMOylation inhibition to reactivate immune responses. Finally the current set of potential options for inhibition of the SUMOylation cascade are discussed, targeting the SAE1/UBA2 heterodimer, E2 UBC9 and several SENPs. We discuss the current state of *in vitro* and *in vivo* research and ongoing clinical trials.

Various players in the SUMOylation machinery are deregulated in different types of cancer. Knowledge of the role SUMOylation plays in tumor progression and malignancy would increase our insight in the role of SUMO in cancer and clarify its potential as a therapeutic target. In **Chapter 2** we investigate SUMO in relation to clinical pathogenesis of conventional chondrosarcomas, malignant cartilage forming tumors of the bone. We analyzed three individual chondrosarcoma datasets, showing that high SUMO2/3 expression is associated with decreased survival, specifically related to aggressive subtypes of disease, indicating SUMO2/3 as a potential prognostic biomarker of disease. A set of chondrosarcoma cell lines was sensitive towards SUMOylation inhibition by ML792, a small molecule inhibitor of SUMO E1 enzyme, especially dedifferentiated chondrosarcoma was found to be sensitive. *In vitro* inhibition of SUMOylation interferes with cell division and consequently chromatin bridges are formed between dividing cells.

To broaden our understanding of SUMOylation in cell division, we focused on SUMOylation of the anaphase promoting complex/cyclosome (APC/C) in **Chapter 3**. Here we show that SUMOylation of APC/C results in substantial structural rearrangements of the WHB domain in the APC2 subunit of APC/C. The mitotic checkpoint complex (MCC) inhibits APC/C to ensure bipolar spindle attachment of all kinetochores before releasing the spindle assembly checkpoint (SAC). This MCC binds to the WHB domain of APC2. The structural changes upon SUMOylation reduced the affinity of the MCC for APC/C. This allows the APC/C to be reactivated when the SAC is silenced, ensuring progression to anaphase. The subtle role SUMO plays in anaphase progression revealed a key piece of the SUMO cell cycle puzzle and helps us to understand how SUMOylation inhibition effects cell cycle progression.

Despite the promising *in vitro* effects shown in Chapter 2 we realized that single therapy with SUMOylation inhibition is still limited in its effect. Therefor we set out to search for a fitting combination therapy in **Chapter 4** to strengthen the anti-tumor potential of SUMOylation inhibition. The hypomethylating agent 5-Aza-2' deoxycytidine was found to be linked to SUMOylation. 5-Aza-2' entraps DNMT1 to the DNA, DNMT1 is subsequently SUMOylated and degraded by the proteasome, resulting in hypomethylated DNA. Combining 5-Aza-2' with SUMOylation inhibition prolonged the entrapment of DNMT1 to the DNA, causing DNA damage. Since SUMOylation is important in many DNA damage repair pathways, we found a dual hit mechanism, causing DNA damage and impairing repair. 5-Aza-2' and TAK981 synergized to reduce B cell lymphoma out growth *in vitro* and *in vivo*. Our findings reveal a tailored combination therapy based on insight in the molecular mechanism of both drugs, strengthening the therapeutic potential of SUMOylation inhibition.

Following the synergistic potential of the therapy described in Chapter 4, we continued this line of research now including immune therapy. The latest research in the field of SUMOylation therapeutics focuses on exploiting the immune modulatory potential of SUMOylation inhibition. In **Chapter 5** we reveal a highly effective combination of therapies in which SUMOylation inhibition together with hypomethylating agent 5-Aza-2' potentiates TCR T cell therapy. An acute myeloid leukemia model is targeted with NPM1-TCR T cells as a model system. SUMOylation inhibition alleviates transcriptional repression on the interferon pathway. T cells targeting tumor cells produce interferons that function as activation signals and consequently enhance tumor killing. *In vivo* TAK981 treatment increased TCR T cell persistence and increased TCR T cell activation over time. Upon the combination

treatment of TAK981 with 5-Aza-2', TCR T cells gained *in vivo* proliferative capacity. Furthermore, also tumor antigen presentation was enhanced, allowing for better T cell recognition of tumor cells. Resulting in a unique and strikingly effective combinational therapy.

To conclude, the research conducted in this thesis is summarized in **Chapter 6**. The potential use of SUMOylation inhibition via an E1 enzyme targeted approach to halt cancer progression is reviewed in a broader context and future perspectives are discussed.