Exploring and exploiting cell cycle regulation of CD8+ T cells

Haften, F.J. van

APPENDIX

ENGLISH SUMMARY

Cancer is the second leading cause of death worldwide. Cancer patients are treated with distinct therapies, including radiotherapy, immunotherapy, and chemotherapy of which the latter is being given to 30% of cancer patients. These chemotherapeutic drugs are designed to target rapidly proliferating cancer cells and thereby induce tumor cell killing. While the effects of chemotherapy on cancer cells are well-studied, the effects on other proliferating cells, including immune cells like cytotoxic $CD8^+$ T cells, which have the ability to directly eliminate tumor cells, are not well known yet. Given the importance of $CD8^+$ T cells in the fight against cancer, it is essential that we better understand the effects of chemotherapy on $CD8^+$ T cells.

Upon encountering its cognate antigen, the T cell receptor (TCR) of a $CD8^+$ T cell is triggered, thereby providing the first activation signal. To become properly activated, $CD8^+$ T cells also need to receive costimulation (signal 2). After receiving these two consecutive signals, a $CD8^+$ T cell can enter the cell cycle, allowing to go through distinct phases in preparation for cell division. Multiple rounds of division result in clonal expansion of the activated $CD8^+$ T cell. During expansion, $CD8^+$ T cell populations not only expand but also start to differentiate into rapidly proliferating effector cells producing cytokines (e.g., interferon-gamma and tumor necrosis factor) and cytotoxic molecules (e.g., granzymes and perforins). To improve the process of proliferation and differentiation, cytokine signaling (signal 3) needs to take place. The processes of proliferation and differentiation are both needed to install a large effector cell pool able to kill target cells and eventually develop into memory $CD8^+$ T cells providing lifelong protectivity immunity by

Initiation and continuation of the cell cycle of $CD8^+$ T cells is a tightly regulated process. In **chapter 2**, we review the current knowledge and consensus of the cell cycle regulation of $CD8^+$ T cells and how proliferation is affected by internal and external cues. We provide an overview of how TCR triggering, costimulation and cytokine signaling all influence the regulation of the cell cycle directly and that these three signals determine the outcome of $CD8^+$ T cell proliferation. Furthermore, we elaborate on how the cross-regulation of the cell cycle and the metabolism and how internal cues participate in the metabolism of $CD8^+$ T cells. Finally, we propose that understanding the processes involved in the regulation of the cell cycle can be used to tweak and exploit $CD8^+$ T cell expansion to improve $CD8^+$ T cell-based therapies for cancer.

In **chapter 3** we show with a reductionist approach how short-term inhibition of the cell cycle of $CD8^+$ T cells impact their proliferation capacity and differentiation phenotype. We show unexpectedly that short term inhibition of the cell cycle after activation of $CD8^+$ T cells,

results in increased proliferation and improved effector cell differentiation. We investigated the underlying mechanisms causing this phenotype and we found that the metabolism is differentially regulated in these cells. We found that during a short-term cell cycle arrest $CD8^+$ T cells stockpile nutrients, such as glucose and amino acids. In addition, we showed that after releasing these cells from the short-term cell cycle arrest, glycolysis is upregulated in an mTORC1 independent way. We observed that arrested cells upregulate the production of IL-2, resulting in improved STAT5-mediated downstream IL-2 signaling in released cells. Finally, we translate our findings to an *in vivo* setting, and we show that temporal cell cycle inhibition of $CD8^+$ T cells can improve immunotherapeutic therapies for cancer, including adoptive cell transfers, vaccination and immune checkpoint blockade.

In **chapter 4** we showed that the combination of vaccination and chemotherapy with cisplatin and topotecan improves the anti-tumor killing effect of $CD8^+$ T cells. We observed that both survival and proliferation of $CD8^+$ T cells are positively impacted by this combination treatment, resulting in altered $CD8^+$ T cell kinetics including delayed contraction. In addition, we investigated the impact of this combination treatment on the tumor micro-environment, and we found that myeloid cells enhance expression of the costimulatory molecule CD70, leading to CD70-mediated improved survival of $CD8^+$ T cells in the tumor micro-environment after combination treatment with vaccination, cisplatin and topotecan.

In **chapter 5** we discuss how better understanding of cell cycle regulation in $CD8^+$ T cells is essential for the field of immunology. We discuss how our findings on $CD8^+$ T cell proliferation can contribute to improving T-cell based therapies in the clinic. Furthermore, we give suggestions on how to accomplish this and how future research can contribute to further improve immunotherapy for cancer patients.