Manipulations of the ubiquitin proteasome system and their effects on antigen presentation

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Scope of the thesis

Proteins are targeted for degradation by a family of ubiquitin (-like) molecules. These proteins are covalently bound to target proteins via chaperone molecules and tag the protein for degradation by the proteasome. The proteasome is a large multi-subunit proteolytic complex, which is specialized in recognizing the tagged proteins and subsequently performs unfolding and degradation of the protein to peptides. The peptides are then degraded by other peptidases like tri-peptidyl peptidase II to single amino acids. There are multiple reasons for tagging a protein for degradation. Many proteins are not correctly folded and will be destroyed even before they have been biologically functional. Other proteins may be degraded because the cell does not need them (anymore) and as a consequence of "damage" during their lifespan.

The major histocompatibility complex (MHC) class I route of antigen presentation has evolved after the protein degradation pathway and is incorporated thus for its own use. A minor fraction of the peptides generated by cellular proteases can bind an ER resident transporter named the transporter associated with antigen presentation (TAP). These peptides can bind TAP in a size and sequence dependent manner and are subsequently transported over the ER membrane. With the help of several dedicated chaperones, the peptides can be bound to empty MHC class I molecules which are also located in the ER. A complex, which consists of a MHC class I molecule, a peptide and a protein named beta-2-microglobuline can leave the ER and is transported to the plasma membrane where it can present the bound peptide to cytotoxic T cells (CTLs). In this way the cell is able to present a blueprint of its intracellular protein content to the immune system. Because the MHC class I route employs the protein degradation route, the cell will not only present peptides of self-proteins, but also peptides of foreign intracellular proteins, like viral proteins (in fact, it cannot see the difference).

The problem of most cancer therapies is the non-specific way by which they reach their goal. Surgery, which is the most effective cancer therapy, has been the most specific treatment of local macroscopic tumours. Because a fast amount of tumours cannot be (completely) removed by surgery, additional treatments like radiotherapy, chemotherapy and immunotherapy have evolved. These therapies have a great advantage, which on the other side is also a major disadvantage: they affect many (in most cases innocent) cells besides the primary tumour. The main objective of cancer therapies is to stop proliferation of tumours. Because this can be achieved in many ways, multiple effective therapies have evolved, in some cases however without full understanding about the exact mechanism(s) by which the therapy is effective.

Chapter 1 and **2** give an overview of the MHC class I antigen presentation pathway, including the specific roles of ubiquitin and the proteasome. Multiple studies have shown that MHC class I molecules do not exclusively present peptides derived from intracellular proteins, but also from other (extracellular) origin. These studies have led to various theories on alternative pathways for extracellular antigens to intersect the classical pathway in a process named cross-presentation. We have evaluated the ER-phagosome fusion theory on cross-presentation in **chapter 3** in a mathematical way and suggest that for functional cross-presentation for vaccination, extracellular antigens should find a different route.

Ubiquitin has been studied with the help of many techniques, which were in many cases of biochemical nature. The discovery of the green fluorescent protein has made it possible to study proteins more easily by a variety of techniques, many of biophysical nature. For the studies described in **chapter 4** we have coupled the green fluorescent protein to ubiquitin and were able to visualize the localization and dynamics of ubiquitin during normal conditions and proteotoxic stress like proteasome inhibition and heat shock. We have extrapolated these findings in **chapter 5**, where we review the possibilities of a dynamic ubiquitin equilibrium in the cell and the possible consequences for cellular processes during cellular stress and pahtological conditions.

Radiotherapy is one of the most effective cancer therapies and has been used for many years. Although the main actions of γ-irradiation on cells have been subject of studies for a long time, not all effects of radiotherapy could be explained. In **chapter 6** we discuss the effects of radiotherapy on antigen presentation in target cells and show how this can be combined with immunotherapy to successfully eradicate tumours.