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Not just a protein machine: how ribosomes regulate immune response

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Chapter 1

Scope of this thesis

The ability of cancer cells to avoid immune destruction is one of the hallmarks of cancer [1]. This thesis investigates the role of specialized ribosomes in immune escape, and focuses on tumor visibility and responsiveness to immune cells. Also, I investigate how the presence of pro-inflammatory cytokines such as IFNɣ or TNFα, or anti-inflammatory cytokines such as TGFβ, affect ribosome composition, their effect on the antigen processing and presentation machinery (APP) and consequently, tumor visibility to T cells. Further, I explore how alterations to another RP regulate the peptide pool presented by tumor cells.

The recognition of antigens presented via Human Leukocyte Antigen Class I (HLA I) by $CD8^+$ T cells is a fundamental component of anti-viral and tumor immune response. Antigen processing and presentation entails biosynthesis of antigenic source proteins in the ribosome, proteasomal digestion of proteins into peptides, peptide transport into the endoplasmic reticulum (ER) via the TAP complex and loading of peptides onto HLA I molecules (HLA-A, -B, and -C heavy chains and β2-microglobulin (β2m)). Peptide loading is facilitated by general purpose and dedicated chaperones, and peptide-loaded HLA I complexes are transported to the cell surface via the Golgi complex where they can be surveyed by circulating $CD8^+$ T cells [2].

When $CD8^+$ T cells recognize a presented peptide, an immune response is initiated, including the secretion of pro-inflammatory cytokines such as IFNγ and TNFα. This causes tumor cells to take on an “alert-state” which is characterized by the upregulation of APP components [3], a switch to more efficient antigen presentation (immune-APP), the induction of the immunoproteasome [4-6] and increased chemokine secretion, for example [7]. The net effect is to enhance $CD8^+$ T cell response of viral and tumor peptides [8]. Conversely, anti-inflammatory cytokines like TGFβ do the opposite and act as inhibitors of T cell function [9].

Ribosomes translate messenger RNA (mRNA) into proteins and are an essential part of the APP. Ribosomes are located in the cytoplasm of a cell, or attached to the rough ER. Eukaryotic ribosomes consist of 79 ribosomal proteins (RPs) and four ribosomal RNAs (rRNAs), and translate proteins during three steps: 1) initiation, 2) elongation and 3) termination. To execute its function, the ready-made protein leaves the ribosome and is either folded immediately, or modified in a step called “post-translational modification” (PTM).

Until recently ribosomes were viewed as homogenous population, passively translating mRNAs without significant regulatory function. However, recent evidence clearly shows more variability to the ribosome than has previously been acknowledged [10] and this supports its potential of specialized functions [11-14]. These studies have revealed the existence of

heterogeneous ribosomes, and show how substantially diverse RPs and RNA modifications that make up the ribosome are. Importantly, these heterogeneous ribosome populations have been shown to regulate diverse biological processes, primarily through the translation of specific panels of mRNAs [11, 15, 16].

Chapter 2 explores many published examples of ribosome specialization in cancer, focussing on RPs specifically [17]. This chapter covers a broad range of topics, including RPs in oncogenic signaling, stress response, proliferation and resistance to cell death and senescence, and metastasis. Importantly for my thesis, **chapter 2** also lists several published examples of RPs that, directly or indirectly, might play a role in immune evasion. However, most of these examples do not distinguish between specialized and extra-ribosomal functions. Generally, immune evasion can be the result of many different mechanisms [18], including alterations in the antigen repertoire presented to T cells [19], downregulation of APP components [20-22] and the absence of T cell attracting chemokines [7]. Until 2019, Wei et al. provided the only published example of specialized ribosomes generating antigenic peptides for presentation, and that one RP in particular (eS28) played a significant role in this [23]. However, it is currently unknown whether such a specialized ribosome is part of, and regulated similarly as the APP. It is also unknown which other RPs are important for these ribosome populations, and whether this population of ribosomes can be utilized as an escape mechanism from T cell immunosurveillance.

We started to investigate this in **chapter 3**, and knocked-down (KD) a selection of 6 RPs (shS15, shL6, shL28, shS28, shP1, shL14) in the M026 melanoma cell line. We selected these RPs based on published studies suggesting a role of these RPs in immune response (shL6, shL28, shS28, shP1, shL14) or with no reported role in immune response (shS15) and included a scrambled control (shScr). We then measured the effect of each RP KD on both pan class I surface expression and $CD8^+$ T cell recognition of M026 tumor cells. Using this approach, we found that KD of two of the tested RPs (P1 and uL14) resulted in a substantial decrease in T cell recognition of tumors compared to the control. Based on these results, we decided to explore these RPs further in the following chapters. Particularly, we were interested in how immune escape was mediated mechanistically, especially in the context of APP.

Immune cytokines have significant influence on tumor behaviour, however, it is still not fully understood how these effects are mediated. In my thesis, I explore this from a translational standpoint and study how cytokine-mediated tumor rewiring is regulated mechanistically, through changes in the ribosome. The stoichiometry of RPs has been of particular interest in this context [24, 25], and we hypothesized that ribosomal alterations in RP abundance could

be induced by pro-inflammatory and anti-inflammatory signals, similar to other parts of the APP. In **chapter 4,** we have identified and characterized a cytokine-responsive, P-stalk-containing ribosome population (PSR) that acts as master regulator of cytokine-mediated processes [26]. We found that both pro- and anti-inflammatory cytokines regulate P-stalk incorporation into the ribosome, partly by TGFβ-mediated phosphorylation. P-stalk containing ribosomes preferentially translate cytokine-responsive mRNAs important for immune response, including HLA. This also has functional consequences, and loss of P1 significantly reduces $CD8^+$ T cell recognition and killing of tumor cells.

In **chapter 5**, we have identified that loss of another RP, uL14, substantially affects both APP and $CD8^+$ T cell recognition of tumors. Specifically, our data show that peptides generated in uL14 KD tumor cells have different characteristics compared to control cells, including lower predicted binding to HLA alleles and a shorter predicted HLA-peptide complex half-life. Functionally, we found that uL14 KD in tumor cells results in decreased $CD8^+$ T cell recognition and killing, potentially as the result of alterations in the peptide pool available for presentation, and this mechanism has previously been reported [27]. Unlike the P-stalk, uL14 association with the ribosome does not change after cytokine exposure suggesting that it is not part of a cytokine response.

Ribosomes perform a crucial biological function, and understanding how they work is pivotal. Ribosome specialization is an extremely novel field and is developing rapidly, however, there are still many open questions to be addressed. This is partly because of how challenging it is to study ribosome specialization technically, and the limited availability of literature on the topic. In **chapter 6**, I will discuss the relevance of my findings and their limitations, and analyze them in the context of other recently published literature. The ribosomal P-stalk has been of particular interest in the field recently, and a handful of articles, including ours, are starting to uncover its role in diverse biological processes. Further, I will explore additional experimental approaches that could potentially help to unwind the complex role of ribosomes in immune response.

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