Molecular approaches to identify cancer T cell antigens and improve immunogenicity
Hos, B.J.

Citation
Hos, B. J. (2023, June 29). Molecular approaches to identify cancer T cell antigens and improve immunogenicity. Retrieved from https://hdl.handle.net/1887/3628370

Version: Publisher's Version
License: Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden
Downloaded from: https://hdl.handle.net/1887/3628370

Note: To cite this publication please use the final published version (if applicable).
Appendices

English summary
Nederlandse samenvatting
日本語要約
Dankwoord
Curriculum Vitae
List of publications
English summary

Pivotal advances are made in the field of tumor immunology to develop novel vaccine platforms which can target cancer antigens. While initially such cancer antigens were tumor-associated ‘self’ antigens and antigens of oncoviral origin, clinical application resulted in limited success. Mutations in the genome of tumor cells offers the possibility to identify ‘foreign’ tumor-specific antigens which are more likely to produce effective immune responses due to an absence of tolerance while limiting autoimmune-related side effects. Thanks to the availability of DNA and RNA sequencing techniques, the expression of tumor-associated genes and mutations can be read in advance, thus providing opportunities for the development of personalized vaccines. Several recent clinical studies have achieved important advancements to this approach.

The work described in this thesis has contributed to the field of tumor-specific vaccines in a several ways. It is currently challenging to identify the sequences which have the greatest chance at inducing an immune response. We have developed novel methods in the identification of epitopes for helper T cells, which are necessary for optimal cytotoxic T cell function against cancer and contribute to an immunostimulatory environment in the tumor and lymph nodes. Similarly, optimal vaccine formulation plays a critical role for the induction of T cell responses. Analytical technologies are lacking for gaining a solid understanding of immunological processing of these formulations. These subjects have been addresses in the chapters of this thesis.

A ‘wet’ approach through analysis of eluted MHC bound peptides has been examined in chapter 2 supported by in silico prediction of MHC class I binding. By extracting MHC proteins and their bound peptides from tumor cells, mass spectrometry could be used to identify the sequences of these presented peptides. Not only did this lead to the identification of peptide binding patterns of MHC, it also identified a set of presented mutations which could be used in a therapeutic vaccine approach to improve immunological protection against cancer. In addition, this method reduced the number of candidate peptides from several hundred predicted binders, to eight peptide sequences; drastically increasing the chance of identifying relevant CD8 T cell epitopes.

It is well appreciated that optimal cytotoxic T cell responses are critically dependent on the simultaneous activation of stimulatory helper T cells. Identification of epitopes for cytotoxic T cell responses has been relatively straightforward due to their recognition of MHC class I bound epitopes which need to apply to a strict set of amino acid sequence residues to bind an MHC class I binding groove. Identification of helper T cell epitopes has been more challenging due to more flexible peptide binding rules of the MHC class II binding groove. In silico prediction approaches have therefore been insufficient. Application of the wet approach seemed well-suited for the identification of epitopes bound by MHC class II. Unfortunately, most tumors are MHC class II negative as MHC class II expression is limited to immune cells. In chapter 4, this problem has been addressed by the transfection of tumor cells with class II transactivator (CIITA); a master regulator of the class II presentation mechanism. This resulted in stable expression of the MHC class II presentation machinery in several tumor cell lines of both human and murine origin. Mass spectrometric analysis of peptides presented in MHC class II purified from these transfected cells appeared reliable in the identification of novel epitopes in self antigens, as well as neopeptides containing amino acid mutations. Immunogenicity rate of these identified peptides was high in vaccination studies. Several of these peptides improved tumor protection by vaccination as such or mixed with CD8+ T cell epitopes.

Preclinical research in tumor immunology is frequently conducted with tumor models that have been present in the scientific community for decades. Genetic drift is known to result in genomic alterations in the original cell line present in different research groups. This poses a challenge regarding the interpretation and comparison of the expression of mutation-based
and shared antigens in cell lines from different origins. In Chapter 3, this was made clear by the mutanome comparison of two independent MC38 tumor cell lines. The mutations relevant for the cytotoxic T cell response in chapters 2 and 4 were absent in a MC38 cell line acquired through a public source. The lack of these mutations and the presence of other mutations has implications for interpreting studies using immunotherapy, and makes a comparison between different reports difficult. This finding underscores the importance for reporting genomic characterizations of cell lines used in publications.

Chapter 5 reviews several molecular methods used to increase immunogenicity of cancer-specific vaccines. Molecular modifications of antigens aim to improved targeting, uptake, cross-presentation and maturation of dendritic cells (DCs). Conjugation of ligands and antibodies specific for receptors on DCs have improved the targeting of antigens to (cross-) presenting DCs. In addition, conjugation of a ligand for danger- or pathogen associated molecular pattern receptors, combines the antigen with a maturation signal to the epitope-processing DC, resulting in enhanced DC maturation and T cell activation. Formulations to encapsulate or integrate antigens in liposomes, nanoparticles, hydrogels, or nanostructures are novel approaches that significantly improve vaccine efficacy. Their activity contributes to managing the solubility and delayed release of physicochemical diverse peptides and improved consistency of vaccine administration.

Investigating the processing and presentation of different vaccine modalities by DCs rely on techniques using covalently bound probes, such as fluorescent molecules, for tracking antigen. Relative to peptides, the size and molecular characteristics of such a probe results in a significant change in physicochemical properties and is known to affect processing and presentation. In addition, these markers are generally not stably conjugated to allow tracking in the entire intracellular pathway. Novel techniques are required that are easily applied in studies with differing vaccine formulations and antigens.

Two-step bioorthogonal chemistry used in chapters 6 and 7 is a potential method to interrogate peptide processing by ligation of probes in situ. Required molecular modifications to the antigen are small and can have little reactivity during the biochemistry of processing and binding to MHC. A paired molecular modification in the probe to the modification in the antigen results in a selective reaction preference and covalently links the probe to the antigen. In chapter 6, a copper(I)-catalyzed Huisgen cycloaddition (ccHc) was found effective for the quantification of MHC-presented epitopes by in situ ligation of a fluorophore. However, this reaction requires a copper catalyst, which necessitates the fixation of the cell and prevents live-cell tracking. In chapter 7, the Diels-Alder method was applied, in which a reaction occurs spontaneously between the inserted molecular groups. This reaction provided positive results in vitro and looks promising for future studies to lower background signal during in situ ligation, allowing antigenic peptide tracking in antigen presenting cells.