Probing proteasome activity and function : cancer diagnostics and mechanism of antigen processing

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PROEFSCHRIFT

ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,
op gezag van Rector Magnificus prof.mr. P.F. van der Heijden,
volgens besluit van het College voor Promoties
te verdedigen op dinsdag 5 oktober 2010
klokke 13.45 uur

door

Celia Rosita Berkers

geboren te Amsterdam
in 1980

Promotiecommissie

Promotores:	Prof.dr. H.S. Overkleeft Prof.dr. J.J. Neefjes
Copromoter:	Dr. H. Ovaa
Overige leden:	Prof.dr. T. Schumacher Prof.dr. J. Brouwer Dr. E. Reits Dr. R. Wolthuis

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The work described in this thesis was performed at the Divisions of Cellular Biochemistry and Cell Biology II of the Netherlands Cancer Institute, Amsterdam, The Netherlands. Financial support was provided by the Dutch Cancer Society (KWF), grant nr. NKI 2005-3368, the Koninklijke Hollandse Maatschappij der Wetenschappen and Stichting Fonds Doctor Catharine van Tussenbroek.

Publication of this thesis was financially supported by the Dutch Cancer Society (KWF) and the Netherlands Cancer Institute. Printed by Wöhrmann Print Service, Zutphen.

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Preface

In cells, proteins are continuously synthesized and degraded to control protein levels and thereby regulate a wide variety of biochemical processes. The proteasome is the main cellular degradation machinery, responsible for the degradation of key proteins involved in the regulation of a wide range of cellular processes, including quality control, cell cycle progression, cell differentiation, signal transduction and apoptosis. In addition, the proteasome is involved in immunity. A broad repertoire of antigenic peptides is presented at the cell surface to the immune system by major histocompatibility complexes (MHC) class I, enabling the immune system to monitor changes in intracellular protein content and sense malignant transformation or the presence of viral proteins. Recognition of foreign or abnormal peptides by CD8+ cytotoxic T cells ultimately results in the elimination of the antigen presenting cell. Both foreign and self MHC class I antigens are generated by the proteasome.

The proteasome has been identified as an attractive pharmacological target. Inhibition of the proteasome causes disruption of many regulatory processes, eventually leading to cell death. The observation that many cancer cells are more sensitive to proteasome inhibition than normal cells has led to the development of several proteasome inhibitors for the treatment of cancer. The drug velcade® with the proteasome inhibitor bortezomib as its active ingredient is used for the treatment of multiple myeloma and mantle cell lymphoma, while many clinical trials in other malignancies are ongoing. The clinical success of bortezomib therapy has initiated the development of several second-generation proteasome inhibitors, which differ in their mode of inhibition and downstream mechanism of action. With several proteasome inhibitors becoming available for clinical use, the outstanding challenge is to define optimal treatment regimes and to choose the most suitable proteasome inhibitor for treatment.

Proteasome activity in its broadest sense is the central theme of this thesis. In **chapter 1**, we describe the development and characterization of different chemical proteasome activity probes that can be used to study proteasome activity in a wide range of tissue types using a variety of assays. With the proteasome emerging as a therapeutic target for cancer treatment, accurate tools for monitoring proteasome activity are in demand. Most methods however require prior cell lysis before activity measurements can be performed, or can only be used in genetically modified cells or organisms. **chapter 1.2** describes the development of a dansylated proteasome activity probe and SDS-PAGE based assays to monitor proteasome activity. This probe is the first in its class that could be used to study the activity of velcade® in intact cells conveniently without the need for laborious radiolabeling procedures. In **chapter 1.3**, fluorescent versions of this probe are described that allow a direct readout in SDS-PAGE based assays, circumventing the need for an antibody, while they can also be used for confocal microscopy and flow cytometry-based assays

to detect proteasome activity in cells directly.

Chapter 2 focuses on different uses of these fluorescent proteasome activity probes. We assess the effects of different proteasome inhibitors in preclinical and clinical studies and use these probes to purify proteasome and characterize its activity and composition. In **chapter 2.1**, we compare the effects of bortezomib and the novel proteasome inhibitor CEP-18770 in mouse tissues and in cell lines with acquired resistance to bortezomib due to a specific mutation in one of the proteasome active sites. In **chapter 2.2** we study the effects of marizomib therapy on patient blood samples and in the following chapter (**2.3**), we describe an activity-guided proteasome purification and determine the relative subunit composition in purified bovine liver and spleen proteasomes by mass spectrometry.

Proteasome activity is studied on a molecular level in **chapter 3** and the role of the proteasome in immunity is addressed. Recent studies have identified novel MHC class I epitopes in which two distant parts of a protein are excised and ligated together to form a novel peptide. These non-contiguous antigens are thought to be produced by the proteasome via a transpeptidation mechanism. In **chapter 3.2**, the molecular mechanism of proteasomal antigen splicing is studied, and rules that predict proteasomal antigen splicing are deduced. In contrast to current thoughts we show that this process can be remarkably efficient. Finally, in **chapter 3.3**, we show that proteasome splicing can produce a novel type of antigen, containing an isopeptide linkage.