Antibody-free LC-MS/MS protein analysis of TRAIL

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Chapter 1

Introduction and scope of the thesis

Uncontrolled tumor growth is a characteristic feature of malignancy, which is related to deregulated apoptosis.[1,2] A strategy of interest for cancer drug discovery is restoring apoptosis in tumor cells leading to controlled, programmed cell death while minimizing cytotoxic effects on healthy tissues. Wild type recombinant human Tumor necrosis factor-Related Apoptosis-Inducing Ligand (rhTRAIL) and death-receptor-specific variants are promising in this respect and are currently being developed as novel anti-cancer biotherapeutics.[3–6]

TRAIL induces the extrinsic pathway of apoptosis by triggering death receptors DR4 and DR5 with a strong preference for tumor cells over healthy cells. TRAIL interacts not only with the two death receptors DR4 and DR5, but binds also to three decoy receptors DcR1, DcR2 and OPG. Since the decoy receptors do not have a functional death domain required to form the so-called death inducing signaling complex (DISC), they are unable of transmitting an apoptotic signal and thus neutralize the apoptotic activity of TRAIL. The presence of decoy receptors was the presumed reason that the efficacy of chemotherapy was not improved in phase I and II studies of patients that were treated with rhTRAIL (Dulanermin). Still, these studies showed that rhTRAIL had no additional toxicity or adverse effects.[7–11] A strategy to improve efficacy could be the design and optimization of DR4- and DR5- specific rhTRAIL variants, which showed promising results in cancer cells (*in vitro*) and in tumor-bearing animals (*in vivo*).[12–15] To develop such variants, it is necessary to have variant-specific quantitative bioanalytical methods.

Currently, the analytical golden standard for the quantification of biopharmaceuticals is the enzyme-linked immune-sorbent assay (ELISA), but LC-MS/MS is being developed into a more and more attractive alternative.[16–18] The reasons for this are that ELISAs require extensive method development and that the antibodies used may not be specific, which could lead to inaccurate and possibly poorly reproducible results.[19] Even though ELISA is currently still more sensitive than LC-MS/MS, this picture is changing due to developments in instrumentation. ELISAs also suffer from a lower precision than LC-MS/MS and a rather limited linear dynamic range.[20,21]

Scope of the thesis

The research described in this thesis focuses on the development of targeted LC-MS/MS methodologies to support quantification of different forms of TRAIL in biological fluids. The main theme of method development was the use of generic, antibody-free sample preparation strategies, making use of the physico-chemical properties of the protein/peptides of interest.

Chapter 2 provides an introduction to the use of antibody-free workflows for LC-MS/MS-based protein quantification by reviewing the scientific literature. Limitations and advantages of the antibody-free approach are discussed and compared to conventional antibody-based workflows.

Chapter 3 presents an antibody-free LC-MS/MS method that is able to discriminate wild type recombinant human TRAIL (rhTRAIL) from its closely related DR4-specific variant rhTRAIL^{4C7} reaching a lower limit of quantitation (LLOQ) of 20 ng/mL for both forms in 100 µL of human and mouse serum. Antibody-free sample enrichment is based on immobilized metal affinity chromatography (IMAC) solid phase extraction (SPE) at the protein level followed by tryptic digestion and quantification of two signature peptides. The method is suitable for supporting pharmacokinetic (PK) studies in mice that are dosed with both rhTRAIL forms simultaneously.

Chapter 4 describes an extension of the above-mentioned methodology with a strong cation-exchange (SCX) SPE step prior to IMAC enrichment and miniaturization to a micro liquid chromatography (µLC) separation. Due to this adjustment, sensitivity is increased to 0.5 ng/mL in human and mouse serum, which is close to the LLOQ of a validated ELISA used in clinical studies. It also offers the option of reducing sample volume to 20 µL, which is beneficial for PK studies in mice.

Chapter 5 describes a methodology to quantify endogenous levels of soluble TRAIL by LC-MS/MS down to the sub-ng/mL level in human saliva and induced sputum. In this approach, next to IMAC at the protein level, SCX enrichment is performed after digestion, at the peptide level. This method proves suitable for supporting biomarker studies with sTRAIL.

Chapter 6 summarizes the results and discusses the future perspectives of antibody-free LC-MS/MS methodologies for protein quantification.

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