Identification and Functional Characterization of Novel Plasma Cell-Specific Surface Antigens in Multiple Myeloma

Dissertation zur Erlangung des akademischen Grades der Doktorin der Naturwissenschaften (Dr. rer. nat.)

Eingereicht im Fachbereich Biologie, Chemie, Pharmazie der Freien Universität Berlin

vorgelegt von

Dipl. Ing. (Biotechnologie) Sabine Friedl

Berlin, Juni 2006

1. Gutachterin: Frau Prof. Petra Knaus

2. Gutachter: Herr Priv. Doz. Ralf C. Bargou

Datum der Disputation: 23.11.2006

Acknowledgements

My deepest gratitude is to my parents **Gabi** and **Diether Friedl**, who always supported and encouraged me in all my decisions. Thank you for never losing trust in me.

I would like to thank **Prof. Dr. Bernd Dörken** for the opportunity to carry out this project in his group at the MDC, Berlin. Thank you also to **Dr. Kurt Bommert** and **Prof. Dr. Ralf Bargou** from the Dörken group (now University of Würzburg) for their supervision and support.

For her readiness to read and appraise this work, I would like to thank **Prof. Petra Knaus**, Department of Biochemistry at the Free University of Berlin.

Thank you to **Dr. Axel Greiner**, Institute of Pathology at the University of Würzburg for performing the immunohistochemistry. For providing the bispecific single chain antibodies, I would like to thank **Dr. Peter Kufer** and **Micromet AG**, Munich.

I am thankful to all members of the **Dörken lab** at the MDC, for the friendly and sociable working atmosphere. I am especially grateful to **Andreas Lietz** for providing me with loads of good music and many opportunities to laugh when I rather felt like crying and most importantly for saving me countless times from a computational "Super-GAU". Thank you also to **Dr. Martin Janz** for various helpful discussions. For their technical support, I would like to thank **Ute Nitzschke** (MDC, Berlin) and **Daniela Dietrich** (University of Würzburg).

I greatly appreciate that my **friends** were not deterred by my frequent mood-swings during the past 4 years. Sometimes there was a lot they had to put up with! Thanks for sticking by me nonetheless.

Finally, my special thank is to **Dr. Anne Mayer-Scholl** and **Dr. Carsten Poetzsch** for being such good friends. Thank you for many pleasant stays in Tramnitz, for looking after Lara so many times, and for reminding me that there is a life outside the lab.

Abstract

Multiple myeloma (MM) is a clonal B-cell tumour of differentiated and usually slowly proliferating plasma cells, mainly located in the bone marrow. MM is still an incurable disease with a median survival of about 3 to 5 years, and it is responsible for about one percent of all cancer-related deaths in Western countries. However, the precise molecular events causing multiple myeloma are still not fully understood.

Immunotherapeutic approaches are playing an increasing role in the development of novel treatment strategies of various malignancies. In particular, the use of recombinant bispecific single-chain antibodies (bssc-ABs) is gaining in importance, because these molecules possess exceptional biological properties. However, to date the lack of suitable plasma cell-specific surface antigens has hindered the development of antibody-based treatment strategies for MM. In order to identify such plasma cell-specific antigens hybridoma supernatants (generated by Dr. Axel Greiner, University of Würzburg) were screened by flow cytometry with a panel of human multiple myeloma-, plasma cell leukaemia-, and B-cell lymphoma cell lines. Three supernatants were found reactive with human MM cell line RPMI-8226. Western blot analysis revealed a band of ~25kDa. A single specific spot was identified with two-dimensional SDS-PAGE and Western blotting of RPMI-8226 membrane proteins and this protein-spot was further analysed by MALDI/MS. Database comparison of the peptide sequence identified the putative plasma cell-specific antigen as human lambda (λ)-light chain.

Recently a novel monoclonal antibody (mAB), designated anti-Wue-1, has been generated which specifically binds to the cell surface of normal and malignant human plasma cells (PC) and mucosa-associated lymphoid tissue (MALT) lymphoma with PC differentiation. On basis of the anti-Wue-1 mAB, a novel MM directed recombinant bispecific single-chain antibody was engineered, designated bssc-anti-Wue-1xCD3 or MT105. Part of this project was to analyze the biological properties of bssc-anti-Wue-1xCD3, using the MM cell line NCI-H929 co-cultured with effector T-cells, isolated from buffy coats of healthy donors. It was demonstrated that bssc-anti-Wue-1xCD3 induces efficient T-cell mediated cell death of NCI-H929 cells. In contrast to conventional bispecific antibodies, bssc-anti-Wue-1xCD3 was efficacious at low effector to target (E:T) ratios and without any additional T-cell stimulation.

The third part of this PhD project was dedicated to the identification, cloning and functional characterization of the novel WUE-1 antigen. First, WUE-1 positive MM cell lines were

identified, using flow cytometry. However, further characterization of the antigen by standard methods such as Western blot analysis or immunoprecipitation kept failing. Subsequently, the attempt was undertaken to isolate the antigen by means of expression cloning and immunoselection with anti-Wue-1 mAB ("panning"). The isolated clones were analyzed by sequencing, Northern blotting, and flow cytometry, but the clones turned out to contain only non-specific inserts.

Since the efficacy of the single-chain (sc) anti-Wue-1 antibody and its advantages over the parental mAB were successfully shown, a recombinant chimeric T-cell receptor (TCR) was generated, comprising the variable single chain (Fv sc) anti-Wue-1 antibody domain, attached to a human IgG Fc sequence, the transmembrane CD28 moiety, and an intracellular CD3ζ signalling domain. This WUE-1 specific TCR was used to develop a novel assay for use in a multiple myeloma expression library screen. Jurkat (T-) cells transfected with the chimeric TCR served as bioindicators. Specific TCR crosslinking with an antigen results in MHC-independent effector cell activation, which can be monitored by cytokine ELISA. Here, expression of the WUE-1 specific TCR on grafted Jurkat cells was successfully demonstrated by triggering the interferon gamma (IFN-γ) release by addition of Fc specific anti-human IgG antibody. However, IFN-γ could not be detected in co-cultures of transfected Jurkat effector cells and WUE-1 positive target cells.

Although the molecular structure of the WUE-1 antigen is still unclear, it was shown that its expression profile and biochemical characteristics discriminate WUE-1 from other plasma cell-associated antigens described so far. Moreover, it was demonstrated that bssc-anti-Wue-1xCD3 induces efficient T-cell mediated cell death at low E:T ratios, and without any additional T-cell stimulation. WUE-1 therefore represents a very promising candidate for use in the development of novel immunotherapeutic treatment strategies of multiple myeloma.

Index