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Untersuchung der Epitopspezifität klonierter Antikörper aus Tumor-infiltrierenden Makrophagen

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Recent evidence from our laboratory has shown a minor subpopulation of monocytes and macrophages expressing somatically recombined immunoglobulin heavy and light chain genes and has resulted in the report of the first fully functional macrophage-derived antibody – designated DER1G10/20 – derived from a single tumour-associated macrophage. The aim of this study was to identify the antigen specificity and get a first insight into the function of this antibody expression by macrophages in the tumour microenvironment.

For this purpose, human blood cells, tissues and cancer cell lines were investigated to identify the respective target antigen by immunohistochemistry, FACS and Western blotting. FACS analyses revealed that the first recombinant macrophage-derived antibody reacts with an intracellular antigen in PBMCs and human cell lines. Further, Coomassie staining revealed the specific precipitation of a 35 kDa protein band from PBMC lysates. To identify the target antigen, immunoprecipitated proteins from various cell and tissue lysates were analyzed by nanoLC-MS/MS mass spectrometry. Statistical evaluation of the mass spectrometry data indicated that Stomatin was the most frequently precipitated specific protein among all samples, although several other candidate proteins were detected by this approach. To confirm a direct interaction, recombinant Stomatin was precipitated with the macrophage-derived antibody from solution. Subsequently, it was verified by RT-PCR and Sanger sequencing that non-mutated Stomatin mRNA is expressed in the tumour tissue where the macrophage antibody was originally isolated from.

Together, initial experimental findings with the macrophage-derived antibody are consistent with the mass spectrometric identification of Stomatin, an intracellular scaffolding protein, as target antigen. However, the function of this anti-Stomatin autoantibody regarding tumour growth and anti-tumour immunity remains unclear. Also the role of Stomatin in cancer is unknown and requires further investigations to determine altered expression levels and the prognostic value in malignant tumours.

Collectively, this study demonstrates that tumour-associated macrophages are capable of producing antibodies with somatically recombined and mutated variable regions directed against self-antigens which are expressed in the tumour tissue. To my knowledge, this is the first report determining the antigen specificity of an innate immune-cell derived immunoglobulin, which challenges the current concept of innate and adaptive immunity and might spur future investigations in this field of research.