Chapter 7

**English summary**

Multiple sclerosis (MS) is a chronic, neurodegenerative disease of the central nervous system (CNS) characterized by inflammation, blood brain barrier (BBB) dysfunction and axonal loss. Although the cause of MS is still not elucidated, both environmental and genetic factors have been proposed to play a role in MS onset. MS is considered the most frequently acquired neurological disease leading to permanent disability in young adults. The majority of the patients (ca 85%) display a relapsing-remitting (RR) course of the disease in which episodes of neurological disability are followed by periods of remission. In time, 60-70% of RR patients progress into a disease phase characterized by progressive neurological decline, termed secondary-progressive (SP)MS. Around 10-15% of the patients develop primary-progressive (PP)MS, a disease form characterized by a continuous worsening of the clinical symptoms from disease onset without remission episodes. Neurological and cognitive evaluation as well as magnetic resonance imaging (MRI) are currently used to diagnose and monitor the progression of the disease. Nevertheless, the severity and clinical course of MS are extremely variable among patients and largely unpredictable. Although during the last decades new insights have been gained in understanding and characterizing MS pathology, to date only few reliable biomarkers are available in the clinic. Therefore, there is still an unmet need for the identification of biomarkers that predict disease activity and progression. In addition, although several drugs are available to reduce inflammatory relapse frequency and accumulated disability, to date there are no effective cures. Therefore, there remains a high need for the discovery of more specific targets and the development of more effective drugs, primarily aimed at modulating disease progression. Although the pathophysiology is not well understood, MS results in infiltration of peripheral blood mononuclear cells (PBMCs) (mainly monocytes/macrophages) into the CNS parenchyma where myelin antigens are recognized and attacked. Upon activation by inflammatory mediators, monocytes adhere to brain endothelium via adhesion molecules, migrate through the inflamed blood brain barrier and differentiate into macrophages in the brain parenchyma. Depending on the inflammatory milieu, macrophages will adopt either a pro- or anti-inflammatory phenotype thereby contributing to either tissue damage or tissue remodeling, respectively. In particular, anti-inflammatory macrophages have been shown to play a role in the removal of myelin debris thus contributing to reduction of inflammation and tissue remodeling.

Tissue Transglutaminase (TG2), a calcium-dependent enzyme that catalyzes cross-linking between proteins, is the best characterized among the Transglutaminase family. In addition, it has various other enzymatic functions, i.e. disulfide isomerase function, G protein (GTPase) function and protein kinase activity. Despite being located primarily in the cytoplasm, TG2 can also be found associated with the cell surface and with the mitochondrial or nuclear membrane. Furthermore, it can also be secreted into the extracellular matrix (ECM) through a non-canonical pathway. TG2 is constitutively expressed in many tissues and cell types, amongst others in macrophages, and is up-regulated in a cell type-dependent manner by
several physiological and pathological stimuli. Because of its diverse functions, its expression and enzymatic activity are strictly regulated in the cell and TG2 dysfunction is associated with several human pathologies.

Previous research has indicated that TG2 plays a role in MS pathology. In particular, our group showed that TG2 is expressed in infiltrating MHC-II positive cells in brain parenchyma of MS patients, while it was absent in the brain parenchyma of healthy control (HC) subjects. In addition, inhibition of TG2 activity in an animal model of MS reduced clinical symptoms and attenuated the influx of monocytes/macrophages into the CNS. The studies presented in this thesis aimed to expand our current knowledge on TG2 in MS and to focus on TG2 expression and function in vitro, and potential clinical implications in MS patients.

For this purpose, we first determined the expression and function of TG2 in human monocytes and macrophages. In particular, in chapter 2 we investigated the expression of TG2 in MS patient-derived monocytes compared to HC subject-derived monocytes. We observed higher TG2 mRNA levels in monocytes from MS patients and a positive correlation between TG2 mRNA levels and anti-inflammatory markers. In addition, we showed that IL-4 is a major inducer of TG2 in human monocytes, where TG2 contributes to adhesion/migration and enables an anti-inflammatory phenotype of the cells. On the basis of these outcomes, we hypothesized that circulating IL-4 could be responsible for the elevated TG2 expression and anti-inflammatory phenotype in MS patient-derived monocytes compared to HC subjects.

Subsequently we focused on macrophage-derived TG2 in chapter 3. We showed that, alike human monocytes, TG2 is selectively up-regulated by IL-4 in human monocyte-derived macrophages. In addition, inhibition of TG2 expression in IL-4 polarized macrophages (M(IL-4)) leads to a pro-inflammatory phenotype and to an impairment in phagosome maturation upon myelin phagocytosis. Based on those results we propose a novel, potentially beneficial function of TG2 in the removal of myelin debris by M(IL-4) macrophages.

In the second part of the thesis, we explored the potential clinical implications of PBMC-derived TG2. In particular, in chapter 4 we investigated whether PBMC-derived TG2 mRNA could be a candidate biomarker for MS activity and/or progression. We observed that TG2 mRNA is positively associated with the patient’s disability status, (Expanded Disability Status Scale (EDSS)), while being negatively associated with normalized brain volume (NBV) and both normalized white matter volume and normalized grey matter volume (NWMV and NGMV, respectively) in MS patients as group and especially in PP-MS patients. In addition, in RR-MS patients, TG2 predicted the change in EDSS over a two-year follow-up thus highlighting also its predictive value. We propose that PBMC-derived TG2 may holds promise for use as biomarker for disease progression in MS and especially in PP-MS patients.

Besides full-length TG2 (V1), four shorter splice variants (V2, V3, V4a and V4b) have been described in the literature with proposed different functions and activity compared to full-length TG2. Based on the results obtained in chapter 4, in chapter 5 we questioned
whether TG2 could have additional value as biomarker for MS diagnosis, specifically in the PP subgroup of MS patients. We observed that the expression of splice variant V4b as well as the percentage of expression (over V1 full-length TG2) of two splice variants (V4a and V4b) was significantly higher in PP-MS patients compared to HC subjects. Thus, we suggest that the expression of TG2 splice variants may be of pathophysiologic relevance in PP-MS patients. In addition, we speculate that the expression pattern of certain TG2 splice variants in PBMCs could be used as potential diagnostic marker to discriminate between HC subjects and PP-MS patients.

In conclusion, we investigated for the first time the role of blood cell-derived TG2 in MS pathology. Through different approaches, ranging from fundamental to translational research, we expanded our current knowledge on TG2 and highlighted the complexity of TG2 biology in the field of MS. Finally, our work opens new avenues to the potential clinical application of TG2 as biomarker for MS disease progression.