RGCG-32, FasL, and SIRT1 as Potential Biomarkers of Relapse and Response to Treatment with Glatiramer Acetate in Multiple Sclerosis

A.M. Kruszewski1, C.A. tegla1,2, C. cudrici1, V. Nguyen3, G.K. Rao1, W. royal1,3, C.T. Bever Jr.1,2,4, V. Rus3 and H. Rus1,2,4

1Dept. of Neurology, Univ. of Maryland School of Medicine, Baltimore, MD; 2Research Service, Veterans Administration Md Health Care System, Baltimore, MD; 3Dept. of Medicine, Univ. of Maryland School of Medicine, Baltimore, MD; 4Veterans Administration Multiple Sclerosis Center of Excellence, Baltimore, MD

Background

Patients with Relapsing-Remitting Multiple Sclerosis (RRMS) are commonly managed with first-line drug treatments glatiramer acetate or interferon beta to reduce but not eliminate the frequency of relapse events over time. However, due to the heterogeneous nature of RRMS, it is difficult to predict patient prognosis and response to treatment. It is critical for the development of reliable biomarkers to aid clinicians in the management of RRMS patients. We have previously shown that the (Acetylamino) in Complement (RGCG-32) is expressed by CD14+ T cells in peripheral blood mononuclear cells (PBMCs) and in brain tissue from RRMS patients [1]. We have also found that RGCG-32 mRNA expression is significantly lower in patients with relapses compared to those in remission and healthy controls [2]. Torgler et al. [3] have recently identified cell cycle regulator [2, 3]. Overexpression of RGCG-32 leads to an increase in DNA synthesis and cell cycle progression from the G1 to S/G2 phase [1]. Both of these responses can be abolished by knocking down the cells with RGCG-32-specific siRNA (A). RGCG-32 forms complexes with cell division cycle protein homolog 2 (CDC2) and enhance CDC2 kinase activity (B). Thus, RGCG-32 appears to be a previously unrecognized regulator of cell cycle involved in cell cycle activation. Despite these findings, little is currently known about the potential role of RGCG-32 in autoreactive T-cells, including RRMS, myelin and myelin-proteolipid oligodendrocytes in the CNS are targeted for autoimmune attack via antigen-specific CD4+ T cells [4, 5]. Several studies have demonstrated impaired apoptosis of T-cells in patients with RRMS. At baseline, the expression of T-cell is regulated by multiple factors, including the CDC2/cyclin-B1 complex [11]. SIRT1, a NAD+-dependent histone deacetylase and key regulator of cell survival and apoptosis, is also regulated in part by the CDC2/cyclin-B1 complex. SIRT1 expression has been found to be decreased in the PBMCs of RRMS patients during relapses [12]. Furthermore, SIRT1 expression was reduced by 50% following RGCG-32 silencing, suggesting an important role for RGCG-32 in SIRT1 expression [12]. Since RGCG-32 binds to the CDC2/cyclin-B1 complex and up-regulates its activity, it is possible that RGCG-32 is involved in regulating T-cell survival by modulating the expression of Fas and SIRT1.

Objectives

It has been shown in previous reports that RGCG-32, FasL, and SIRT1 mRNA expression is significantly lower in PBMCs of RRMS patients during relapse compared to remission. The present study aims to longitudinally investigate the combined roles of RGCG-32, FasL, and SIRT1 as possible peripheral blood-based biomarkers of relapses and predictions of response to glatiramer acetate (GA) treatment in a cohort of RRMS patients.

Materials and Methods

Patients and controls. A cohort of 15 patients with RRMS were enrolled in the study. The patients were primarily recruited from three centers: Baltimore Multiple Sclerosis Center, the Johns Hopkins Multiple Sclerosis Center, and the University of Pennsylvania. The patients were defined with RRMS according to the revised McDonald criteria [6-8]: (i) fulfillment of McDonald criteria for definite MS; (ii) requiring relapse treatment; (iii) having newly diagnosed MS or not treated with currently used immunomodulating drugs (interferon or glatiramer acetate) for 3 months prior to study entry. The patients had no acute exacerbations in the 4 weeks before the study; (iv) no infections; (v) steroids for 4 weeks prior to study enrollment; (vi) treatment with Trexal, Glucoma, immunosuppressants, cyclosporines, or investigational drugs during the past year; (vii) no history of malignancy except for basal cell carcinomas; (viii) no history of autoimmune disease, cardiovascular disease, blood disorder, diabetes mellitus, renal disorder, or neurologic disorder; (ix) no history of glialencephalitis or melitoidus enteritis; (x) no history of alcohol or drug abuse.

Study design. All MS patients received 20 mg of glatiramer acetate (GA) injected subcutaneously every 24 hours for 2 years. During the study period, patients were clinically evaluated and peripheral blood samples were collected at 0, 3, 6, and 12 months at the time of the relapse diagnosis. Patients with a history of smoking were not included in the study. The patients were divided into two groups: group A (n=7; 75% female) and group B (n=8; 75% female). In group A, 6 patients were diagnosed with RRMS (3 patients with RRMS type I) and 1 patient with RRMS type II. In group B, 3 patients were diagnosed with RRMS (2 patients with RRMS type I) and 5 patients with RRMS type II. The patients were longitudinally evaluated during the follow-up period. The mean duration of the study was 27 months.

Collection of PBMCs, total RNA purification, and qPCR analysis. Peripheral blood mononuclear cells (PBMCs) were isolated from each sample using BD Eos 100 Blood sample and RNA isolation was performed on the same day. Total RNA was purified, denatured, and reverse transcribed using oligo-dT primer to synthesise cDNA for each sample. Real-time qPCR was performed using Mx3000P system and primer sets for: (i) GABA-A receptor subunit gamma-2 (GABRA2); (ii) Glutamate receptor subunit alpha-1 (GRIA1); (iii) GABA-A receptor subunit alpha-1 (GABRA1); (iv) GABA-A receptor subunit alpha-2 (GABRA2); (v) GABA-A receptor subunit alpha-3 (GABRA3); (vi) GABA-A receptor subunit alpha-4 (GABRA4); (vii) GABA-A receptor subunit alpha-5 (GABRA5); (viii) GABA-A receptor subunit alpha-6 (GABRA6); (ix) GABA-A receptor subunit beta-1 (GABRB1); (x) GABA-A receptor subunit beta-2 (GABRB2); (xi) GABA-A receptor subunit beta-3 (GABRB3); (xii) GABA-A receptor subunit beta-4 (GABRB4); (xiii) GABA-A receptor subunit gamma-1 (GABRG1); (xiv) GABA-A receptor subunit gamma-2 (GABRG2); (xv) GABA-A receptor subunit gamma-3 (GABRG3); (xvi) GABA-A receptor subunit gamma-4 (GABRG4); (xvii) GABA-A receptor subunit delta-1 (GABRD1); (xviii) GABA-A receptor subunit delta-2 (GABRD2); (xix) GABA-A receptor subunit delta-3 (GABRD3); (xx) GABA-A receptor subunit delta-4 (GABRD4); (xxi) GABA-A receptor subunit delta-5 (GABRD5); (xxii) GABA-A receptor subunit delta-6 (GABRD6); (xxiii) GABA-A receptor subunit epsilon (GABRE); (xxiv) GABA-A receptor subunit zeta (GABRZ); (xxv) GABA-A receptor subunit omega (GABROW); (xxvi) GABA-A receptor subunit chi (GABRC); (xxvii) GABA-A receptor subunit kappa (GABRK); (xxviii) GABA-A receptor subunit theta (GABRT); (xxix) GABA-A receptor subunit zeta-2 (GABRZ2); and (xxx) GABA-A receptor subunit chi-2 (GABRC2).

References


Acknowledgements

This project was funded through a grant from the Foundation of the Consortium of Multiple Sclerosis Centers/MS Workforce of the future program (to A.M.K.), a Veterans Affairs Merit Award (to H.R.), and a grant from TEVA Neuroscience (to H.R.).

Conclusions

1. RGCG-32, FasL, and SIRT1 mRNA expression is significantly decreased in RRMS patients during periods of clinical relapse compared to remission.
2. RGCG-32 and SIRT1 mRNA expression is significantly increased overall in responders to glatiramer acetate treatment compared to non-responders followed over time.
3. ROC analyses suggest that RGCG-32, FasL, and SIRT1, but not CDC2, could potentially serve as peripheral blood-based biomarkers to help confirm RRMS patient relapse and identify response to glatiramer acetate therapy.