Phosphorylated SIRT1 as a biomarker of relapse and response to treatment with glatiramer acetate in multiple sclerosis

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Background

Multiple sclerosis (MS) is a demyelinating disease characterized by chronic inflammation of the central nervous system in which many factors (genetic and environmental) are involved in the pathogenesis of the disease. Effector T cells, activated by the invasion of antigen-presenting cells, release certain cytokines that play an important role in driving the inflammatory cascade. Genetic and environmental factors are increasingly being implicated as regulatory components of the immune response in MS. Histone protein post-translational modifications have the ability to affect chromatin structure and regulate gene expression. SIRT1 (Silent information regulator 1) is a member of the SIRT protein family, made up of 10 sirtuins. SIRT1 deacetylase catalyses the removal of acetyl groups from a variety of protein substrates, including histones H1, H2A, and H3. In addition, SIRT1 has been found to promote histone H3 lysine 9 (H3K9) methylation, resulting in gene silencing. SIRT1 is involved in the regulation of a number of cellular processes, including telomere maintenance, DNA repair, and angiogenesis. SIRT1 can induce chromatin silencing through the deacetylation of histone lysine residues and can modulate the tissue growth by regulating the metabolic activities of α5, NF-kB, FOXO proteins, and p21. Recently, SIRT1 has been shown to be involved in the disease course in MS and to be a target for therapeutic agents. Various studies have shown that the inhibition of SIRT1 enhances the symptoms of MS and can lead to the development of new therapeutic agents.

Results

It has been shown in previous studies that SIRT1 mRNA expression is significantly lower within PBMCs of RRMS patients than control subjects. In this study, we investigated the role of SIRT1 as a possible peripheral blood biomarker of relapse and prediction of response to treatment. SIRT1 is a nutrient-sensing deacetylase that catalyzes the deacetylation of NAD+ and is involved in the regulation of genomic stability, protein stability, metabolic homeostasis, and neuroprotection. In this study, we investigated the role of phosphorylated SIRT1 (p-SIRT1) and imprinted H3K9me3 as biomarkers of relapse and as predictors of response to GA treatment in RRMS patients.

Objectives

Patients: A cohort of 15 patients with RRMS were enrolled in the study. The patients were primarily recruited from the University of Maryland Multiple Sclerosis Center. The criteria for inclusion of MS patients in the study were: (i) age 18 to 60 years; (ii) fulfilment of McDonald criteria for definite MS; (iii) relapsing-remitting course; (iv) having new disease activity in MS, or MS not controlled with currently used immunomodulatory drugs (interferon-β or glatiramer acetate) for 3 months prior to study entry; (v) no treatments in the previous 4 weeks before study; (vi) no corticosteroid treatment or steroid washout for 1 week prior to study enrollment; (vii) no treatment with Tybaltin, Gileten, mycophenolate, or investigational drugs during the past year; (viii) a disease score of 0.5 or defined as the expanded disability status scale (EDSS) ≤ 3.5; (ix) no history of neoplasia; (x) use of antibiotics in the last 30 days; (xi) a history of intravenous or intramuscular or oral corticosteroids in the last 30 days; and (xii) a history of uncontrolled infection.

Study Design: All MS patients received 20 mg of GA subcutaneously every 2 days for 12 months. During this period, patients were already evaluated and peripheral blood samples were collected at 0, 3, 6, and 12 months of the time of their outpatient visits. Patients with symptoms suggestive of a clinical relapse called the University of Maryland Multiple Sclerosis Center. Clinical relapse was defined as sudden worsening of pre-existing symptoms or appearance of new neurological deficits in the absence of fever or infection lasting more than 24 h. An EDSS evaluation was completed at each visit. All patients, clinical symptoms, and infection were reviewed by a neurologist (H.R. C or W.R.) to ensure that the data obtained were complete. In the case of patients with relapses, the admission of 10 to 30% in the EDSS score for 3 days was used to treat the disease exacerbation. A prediction tool was developed by analyzing the relapse rate of patients with RRMS, patients monitored (2 years before GA initiation) and patients were monitored (2 years after GA initiation). The patient was defined as a responder if two or more than 1 relapse events during the year after initiation of GA therapy. GA was considered as a non-responder if two or more relapse events during the year after the initiation of GA therapy. According to these criteria, the present cohort consisted of 12 responders and non-responders to treatment.

Collection of PBMCs: Peripheral blood mononuclear cells (PBMCs) were isolated from each patient's fresh blood sample by ficoll-hypaque gradient centrifugation. The cell pellet was suspended in 80 mL of calcium-free Hanks’ balanced salt solution (Biowest, Haltom City, TX) and stored at -80°C until sample analysis.

Western Blot: Western blot protein analysis was performed using patient PBMC samples that were kept in RNAlater and processed. Whole-cell pellets (protein total, 40-50 μg) were analyzed by 2% SDS-PAGE (Biorad), followed by western blotting. Each membrane was analyzed for p-SIRT1 (phospho-SIRT1-CST), SIRT1 (Abnova), H3K9me3 (CST) using specific antibodies. p-SIRT1 (Rockland) was also used as a loading control for normalization of the different samples. The PVDF membrane was blocked and incubated overnight with primary antibodies against SIRT1, p-SIRT1 and H3K9me3 (CST). p-SIRT1 is a specific antibody used to detect the deacetylated form of SIRT1. The last membrane was rinsed with PBS and incubated with secondary antibodies conjugated with horseradish peroxidase. The blots were imaged using a ChemiDoc MP system (BioRad, Hercules, CA). The band intensity was measured using Quantity One software (BioRad, Hercules, CA). All values are shown as mean ± SEM and are representative of at least three experiments unless otherwise stated.

Statistical analysis: Comparisons between groups were performed using a two-tailed Student’s t test assuming unequal variances. For multiple comparisons, analysis of variance (ANOVA) was used. Statistical analysis was performed using GraphPad software version 6. All values are shown as mean ± SEM and are representative of at least three experiments unless otherwise stated. Receiver operating characteristic (ROC) curve analysis was used to assess the predictive accuracy of each potential biomarker. The predictive probability of outcome reporting clinical state and response to GA treatment was reported as a C-statistic or Area Under the Curve (AUC). AUC was defined as a percentage, with a perfect score being 100% predictive.

Conclusions

1. p-SIRT1 protein levels are significantly decreased in RRMS patients during periods of clinical relapse and significantly increased in patients who are responders to GA treatment.

2. H3K9me3 protein levels are significantly decreased in RRMS patients during periods of clinical relapse.

3. p-SIRT1 protein inversely correlates with phosphorylated SIRT1 protein levels in detecting RRMS patient response to GA.

4. p-SIRT1 could potentially serve as peripheral blood biomarker to help confirm RRMS patient relapse.

5. H3K9me3 could potentially serve as a peripheral biomarker to help confirm RRMS patient relapse and predict response to GA therapy.

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