SIRT1 as a Potential Marker of Disease Activity and Response to Treatment with Glatiramer Acetate in Multiple Sclerosis.

D. Hewes,1 A. K. M. Kruszewski,1 D. Boodhoo,1 A. Tatomir1, V. Nguyen2, G. K. Rao1, W. Royal III3, C. T. Bever Jr.1, V. Rus2,3 and H. Rus1,3

1Dept. of Neurology, Univ. of Maryland School of Medicine, Baltimore, MD; 2Dept. of Medicine, Div. of Rheumatology and Clinical Immunology, Univ. of Maryland School of Medicine, Baltimore, MD; 3Veterans Administration Multiple Sclerosis Center of Excellence, Baltimore, MD

Background

Epigenetic modifiers are increasingly being implicated as regulatory components of expression of neuronal and immune specific genes. Histone protein post-translational modifications have the ability to affect chromatin structure and regulate gene expression. SIRT1 (Sir2; 1) is a member of the HADAC class III family of proteins. It is a NAD-dependent histone and protein deacetylase that regulates the removal of acetyl groups from a variety of protein substrates, including histones H1, H3, and H4. In addition, SIRT1 has been found to promote histone H3 lysine 9 (H3K9) methylation, resulting in epigenetic gene silencing. SIRT1 is involved in the regulation of a number of cellular processes, including transcription, metabolism, DNA repair, and aging. SIRT1 can induce chromatin silencing through the deacetylation of histones and can modulate transcription by regulating the transcriptional activities of p53, NF-kB, FOXO proteins, and p300. Recently, a SIRT1 activator was shown to ameliorate the disease course in experimental autoimmune encephalomyelitis (EAE), a mouse model of MS. Studies have shown that SIRT1 is highly expressed in activated T cells and also to induce a decrease in spinal cord inflammation during EAE. The mechanism for its immunomodulatory and neuroprotective effects involves the activation of SIRT1. SIRT1 expression has also been found to be decreased in the PBMCs of RRMS patients during relapses. Furthermore, SIRT1 expression was reduced by 50% following RGC-32 silencing, suggesting an important role for SIRT1 in RRMS. Little is known about the changes that occur in SIRT1 expression during exacerbation of RRMS patients, which is the role of SIRT1 as a possible biomarker of relapses and as a predictor of response to treatment in RRMS patients.

Materials and Methods

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: age 18 to 65 years; (ii) fulfillment of McDonald criteria for definite MS; (iii) relapsing-remitting course; (iv) no newly diagnosed MS, or MS not treated with currently used immunomodulatory drugs (interferon-β or glatiramer acetate) for at least 3 months; (v) no prior treatment history; and subsequently collected at 3, 6, and 12 months following the initiation of GA treatment. Target gene mRNA expression was measured in patients’ PBMCs by two-step real-time qPCR and expressed as a ratio to L13 mRNA expression. Overall, significantly lower levels of SIRT1/L13 (p<0.003) were found in patients during relapse compared to remission.

Study design

All MS patients received 20 mg of GA injected subcutaneously every 2 days for 2 years. During this period of 2 years, patients were clinically stable. Clinical relapses were diagnosed based on the presence of pre-existing symptoms or appearance of new neurological deficits in the absence of fever or infections lasting more than 24 h. An EDSOS evaluation was completed at each visit. Clinical records, consultation reports, and endpoint records were reviewed by a neuroradiologist to determine the extent of disease. Each patient was classified as responders and non-responders to GA treatment. An example of the western blot relevant to the study design is shown in Figure 1.

Collection of PBMCs

Peripheral blood mononuclear cells (PBMCs) were isolated from each patient’s fresh blood sample at the time of their outpatient visits using BD Vacutainer CPT tubes (Becton Dickinson) and RNA isolation was performed the same day. Total RNA (~0.5 µg per sample) was purified (RNeasy Mini Kit), and reverse transcribed using oligo-dT primers to synthesize cDNA for each sample.

Real-time quantitative PCR

Real-time quantitative PCR was performed using patient mRNA sample cDNA and primer sets specific for SIRT1. For each gene, the cycle threshold (Ct) values were determined in the exponential phase of the amplification curve and normalized to the mRNA expression of L3 ribosomal protein, a housekeeping gene. A standard curve was generated using serial dilutions of reference human total RNA (Clontech), and the normalized mRNA value (NRFV) was calculated according to the following formula for relative expression of target mRNA: NRFV = [Ct(target) - Ct(L3)]/ [Ct(target) - Ct(L3)] p-value and fold change were calculated by the ΔΔCt method.

Statistical analysis

Comparisons between groups were performed using a two-tailed t-test assuming unequal variances. P-values were considered significant. Statistical analysis was performed using SPSS Statistics Software version 22 and GraphPad Prism software version 5. All p-values are given as p<0.05 and are representative of three experiments otherwise noted. Receiver operating characteristic (ROC) curve analysis was used to assess the threshold characteristics of each potential biomarker. The predictive probability of binary outcomes regarding clinical state and response to GA treatment was calculated as a C statistic or Area Under the Curve (AUC), represented as a percentage, with a perfect score being 100% predictability.

Acknowledgements

This project was funded through a grant from the Foundation of the Consortium of Multiple Sclerosis Centers’ MS Workforce of the Future Program (D.H.), and a Veterans Administration Merit Award (to H.R.).

Conclusions

1. SIRT1 mRNA expression is significantly decreased in RRMS patients during relapse compared to clinical relapse comparison. SIRT1 mRNA expression is significantly increased overall in responders to GA treatment compared to non-responders over time.

2. H3K9me2 and H3K9ac expression is correlated to SIRT1 protein level increase in relapses and in non-responders to GA treatment. H3K9me2 expression is significantly decreased in relapses and in responders to GA treatment. In addition, H3K9acme2 is correlated to Fasl mRNA and RGC-32 protein in non-responders to GA.

3. ROC analyses suggest that SIRT1 mRNA could potentially serve as peripheral blood-based biomarker to help predict relapse and response to GA therapy.