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RGC-32 as a potential biomarker of relapse and response to treatment with glatiramer acetate in multiple sclerosis

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ABSTRACT

Currently there is critical need for the identification of reliable biomarkers to help guide clinical management of multiple sclerosis (MS) patients. We investigated the combined roles of Response Gene to Complement 32 (RGC-32), FasL, CDC2, AKT, and IL-21 as possible biomarkers of relapse and response to glatiramer acetate (GA) treatment in relapsing–remitting MS (RRMS) patients. Over the course of 2 years, a cohort of 15 GA-treated RRMS patients was clinically monitored and peripheral blood mononuclear cells (PBMCs) were collected at 0, 3, 6, and 12 months. Target gene mRNA expression was measured in patients' isolated PBMCs by real-time qRT-PCR. Compared to stable MS patients, those with acute relapses exhibited decreased expression of RGC-32 ($p < 0.0001$) and FasL ($p < 0.0001$), increased expression of IL-21 ($p = 0.04$), but no change in CDC2 or AKT. Compared to non-responders, responders to GA treatment showed increased expression of RGC-32 ($p < 0.0001$) and FasL ($p < 0.0001$), and decreased expression of IL-21 ($p = 0.02$). Receiver operating characteristic (ROC) analysis was used to assess the predictive accuracy of each putative biomarker. The probability of accurately detecting relapse was 90% for RGC-32, 88% for FasL, and 75% for IL-21. The probability of accurately detecting response to GA was 85% for RGC-32, 90% for FasL, and 85% for IL-21. Our data suggest that RGC-32, FasL, and IL-21 could serve as potential biomarkers for the detection of MS relapse and response to GA therapy.

1. Introduction

Multiple sclerosis (MS) is an inflammatory-mediated demyelinating disease of the human central nervous system (CNS). The clinical disease course is variable, usually starting with reversible episodes of neurological disability followed by continuous and irreversible neurological decline (Trapp & Nave, 2008). A diverse interplay of immunological factors contributes to a characteristically variable pathology, phenotypic presentation, disease course, and prognosis (McFarland & Martin, 2007). Glatiramer acetate (GA) is one of the most common immunomodulatory therapies used to treat relapsing–remitting multiple sclerosis (RRMS) patients. Results from clinical trials have shown a reduction in the annualized relapse rate of about 29% with this agent (Johnson et al., 1995; Johnson et al., 1998). Unfortunately not all

Table 1
Primers used for quantitative real-time PCR.

Gene symbol	Primer sequence	Product (bp)
RGC-32	For: 5′-AGGAACAGCTTCAGCTTCAG-3′ Rev.: 5′-GCTAAAGTTTTGTCAAGATCAGCA-3′	152
FasL	For: 5′-GCCCATTTAACAGGCAAGTC-3′ Rev.: 5′-ATCACAAGGCCACCCTTCTT-3′	110
CDC2	For: 5′-TTTTCAGAGCTTTGGGCACT-3′ Rev.: 5′-AGGCTTCCTGGTTTCCATTT-3′	100
AKT1	For: 5′-ACGCCAAGGAGATCATGC-3′ Rev.: 5′-CTCCATGCTGTCATCTTGGTC-3′	185
L13	For: 5′-CGTGCGTCTGAAGCCTACA-3′ Rev.: 5′-GGAGTCCGTGGGTCTTGAG-3′	227

For, forward primer; Rev., reverse primer; Bp, base pairs; RGC-32, response gene to complement 32; FasL, Fas ligand; CDC2, cell division cycle protein 2 homolog; L13, ribosomal protein L13. IL-21 primers were purchased from SABiosciences, cat# PPH01684A.

Abbreviations: AKT, AKT8 virus oncogene cellular homolog; AUC, area under the curve; CDC2, cell division cycle protein 2 homolog; CNS, central nervous system; CPT, cell preparation tubes; EDSS, expanded disability status scale; FasL, Fas ligand; GA, glatiramer acetate; IL, interleukin; MRI, magnetic resonance imaging; MS, multiple sclerosis; NRV, normalized mRNA value; PBMCs, peripheral blood mononuclear cells; RGC, response gene to complement; ROC, receiver operating characteristic; RRMS, relapsing-remitting multiple sclerosis; WT, wild type.

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patients respond to treatment, and treatment failure may only be recognized after months or years of therapy. It would be of great value to determine patient responsiveness prior to the selection of a particular MS therapy, including GA. However, with the possible exception of magnetic resonance imaging (MRI), currently there are no markers reliably validated for widespread clinical use in predicting response to MS therapy despite the presence of numerous candidate markers in the serum and cerebrospinal fluid (Furby et al., 2010; Graber & Dhib-Jalbut, 2011; Housley et al., 2015). Thus identification of effective, accessible biomarkers of disease activity and response to treatment would have significant benefits in the clinical management of patients with MS and help prevent further disease progression.

We have identified RGC-32, a novel gene product induced by complement activation, and have demonstrated its activity primarily as a cell cycle regulator (Badea et al., 2002; Badea et al., 1998; Fosbrink et al., 2009). RGC-32 protein forms complexes with CDC2/cyclinB1 and enhances CDC2 kinase activity (Badea et al., 2002; Vlaicu et al., 2008). In addition RGC-32 binds to and modulates the activity of AKT (Fosbrink et al., 2009), and regulates the expression of FasL and interleukin-21 (IL-21) (Tegla et al., 2013). Thus, RGC-32 appears to be a regulator of critical kinases involved in cell cycle regulation and apoptosis. RGC-32 is expressed by $CD3^+$ as well as $CD4^+$ T-cells in peripheral blood mononuclear cells (PBMCs) and in brain tissue from relapsing–remitting MS patients (Tegla et al., 2013). Previous studies have shown significantly decreased

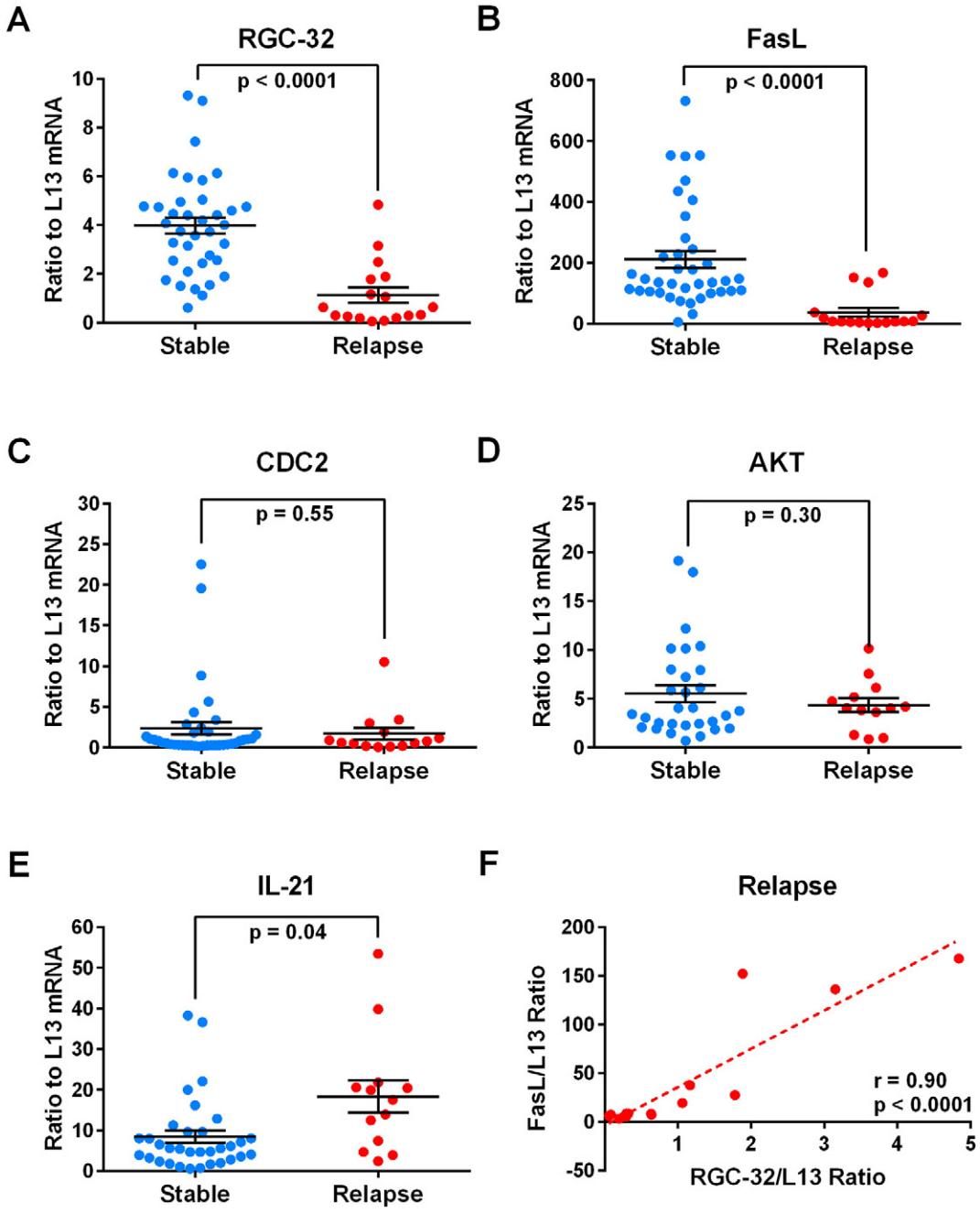

Fig. 1. Expression of RGC-32, FasL, CDC2, AKT, and IL-21 mRNA in stable MS patients and patients with acute relapses. Target gene mRNA expression was measured in patients' PBMCs using real-time qRT-PCR and expressed as a ratio to L13. A. Significantly lower levels of RGC-32 mRNA were found in patients with relapses compared to clinically stable patients ($p < 0.0001$). B. Significantly lower levels of FasL mRNA were found in patients with relapses compared to clinically stable patients ($p < 0.0001$). C and D. No statistically significant changes were observed in CDC2 or AKT mRNA. E. Significantly higher levels of IL-21 mRNA were found in patients with relapses compared to clinically stable patients ($p = 0.04$). F. RGC-32 mRNA expression levels were correlated with those of FasL in patients during relapses ($r = 0.90$, $p < 0.0001$).