Dicer and microRNA expression in multiple sclerosis and response to interferon therapy

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ABSTRACT

Dysregulation of microRNA expression has been shown in multiple sclerosis (MS); however, the mechanisms underlying these changes, their response to therapy and the impact of microRNA changes in MS are not completely understood. Dicer mediates the cleavage of precursor microRNAs to mature microRNAs and is dysregulated in multiple pathologies. Having shown that interferons regulate Dicer in vitro, we hypothesized that MS patient IFNβ1a treatment could potentially alter Dicer expression. Dicer mRNA and protein levels, as well as microRNA expression, were determined in MS patient and healthy control PBL. Acute responses to IFNβ1a were assessed in 50 patients. We found that Dicer protein but not mRNA levels decreases in MS patients while both are selectively induced in patients responding well to IFNβ1a. Potential microRNA biomarkers for relapsing remitting multiple sclerosis (RRMS), secondary progressive multiple sclerosis (SPMS) and IFNβ1a response are described. Contrasts in Dicer and microRNA expression levels between patient populations may offer insight into mechanisms underlying disease courses and responses to IFNβ1a therapy. This work identifies Dicer regulation as both a potential mediator of MS pathology and a therapeutic target.

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1. Introduction

Multiple sclerosis (MS) is a chronic autoimmune/inflammatory disease with a neurodegenerative component affecting the central nervous system. Several reports have suggested microRNA (miRNA or miR) dysregulation in MS patients and attempted to identify miRNAs as biomarkers (Otaegui et al., 2009; Junker et al., 2009; Keller et al., 2009; De Santis et al., 2010; Lindberg et al., 2010; Guerau-de-Arellano et al., 2011, 2012; Waschbisch et al., 2011; Martinelli-Boneschi et al., 2012; Angerstein et al., 2012; Sievers et al., 2012; Jernås et al., 2013; Fenoglio et al., 2013; Hecker et al., 2013; Søndergaard et al., 2013; Gandhi et al., 2013).

MicroRNA are now recognized as an important component of epigenetic regulation and miRNA expression profiles have been found to be disrupted in many pathologies (Esteller, 2011; Ha, 2011; Rottiers and Näär, 2012; Di Leva and Croce, 2013). Most miRNAs are synthesized from RNA Pol II transcripts (primary miRNA or pri-miRs), processed in the nucleus by a type III RNase (Drosha/DGCR8) into shorter hairpin "pre-miRs" which are transported into the cytoplasm by Exportin5. In the cytoplasm, pre-miRs are recognized by another type III RNase, Dicer, and cleaved to their mature ~22 nt form. Mature miRNAs are then incorporated into the miRISC complex of proteins, which includes Dicer, Ago2, TRBP, PACT and other proteins. The miRNA guides the silencing complex to target mRNAs with sufficiently complimentary sequences for either degradation or translational repression (Koscianska et al., 2011).

Currently, 2588 mature miRNAs have been identified in humans and their expression profiles have been shown to be tissue, cell type and developmental stage specific. miRNAs are reported to contribute to the regulation of approximately half of the human genome (Koscianska et al., 2011; Di Leva and Croce, 2013). Alterations in miRNA expression

profiles have been found in many pathologies (Esteller, 2011; Ha, 2011; Rottiers and Näär, 2012; Di Leva and Croce, 2013), particularly those associated with inflammation and immune function (Cobb et al., 2006; Asirvatham et al., 2008; Baltimore et al., 2008; O'Connell et al., 2012; Singh et al., 2013). It has been suggested that miRNA profiles may serve as diagnostic and/or prognostic biomarkers (Weber et al., 2010; Kosaka et al., 2010; Zhang et al., 2012; Chavali et al., 2013). Furthermore, miRNAs are currently under investigation as targets for intervention – with the proposed use of synthetic miRNA mimics or antagonists (antagomiRs) as therapeutic agents (Krützfeldt et al., 2005; Brown and Naldini, 2009; Castanotto et al., 2009; Rupaimoole et al., 2011; Chavali et al., 2013 30–34).

Aberrant expression of several components of the miRNA production pathway has been described in various diseases (Karube et al., 2005; Divekar et al., 2011; Oak et al., 2011; Mockenhaupt et al., 2011; Gascon and Gao, 2012; Sharma et al., 2013). For example, decreased Dicer expression in lung cancer is associated with diminished miRNA expression and poor prognosis (Karube et al., 2005). In MS, overexpression of Dicer mRNA in PBL has been reported (Jafari et al., 2015) while others have shown decreased Dicer protein in B cells from MS patients (Aung and Balashov, 2014). To our knowledge, no studies have analyzed Dicer mRNA and protein expression in the context of complete miRNA profiling. Furthermore, there have not been studies of Dicer expression in MS progression or treatment.

Dicer expression is required for normal development of oligodendrocytes and Schwann Cells (Shin et al., 2009; Bremer et al., 2010). Dicer expression has been shown to be subjected to acute regulation in vitro as well as developmental regulation in vivo (Asada et al., 2008; González-González et al., 2008; Wiesen and Tomasi, 2009; García-López and del Mazo, 2012). Moreover, inflammatory mediators, including interferons and TLR ligands, have been found to cause acute regulation of Dicer in vitro and, interestingly, IFNα and IFNγ may have opposite effects on Dicer protein levels (Wiesen and Tomasi, 2009). Acute regulation of Dicer has not been shown in a therapeutic context and altered expression levels of Dicer in MS have not, to our knowledge, been reported in diagnostic or clinical response groups. Therefore, in this study, we established a population of 100 patients including healthy controls, untreated MS patients and both Good and Poor Responders to weekly intramuscular (IM) IFNβ1a (Avonex®, Biogen Idec, Boston, MA). In these patients' PBL, we determined Dicer mRNA and protein expression together with miRNA profiles in order to support high confidence determination of miRNA expression differences between our study groups.

2. Materials and methods

2.1. Ethics statement

The Institutional Review Boards for both Roswell Park Cancer Institute and SUNY University at Buffalo approved this study and all participants signed approved consent forms.

2.2. Study population

A total of 100 patients and age, sex matched healthy control subjects were recruited to this IRB approved study. Subjects were enrolled in one of four subgroups: 25 healthy controls (HC), 24 MS on no therapy (NT), 50 patients receiving weekly intramuscular IFNβ1a – 25 defined as Good Responders (GR) and 25 as Poor Responders (PR) (Table 1). These 50 patients averaged 18.2 years since diagnosis and had been treated with IFNβ1a for an average of 10.26 years each (Table 1). The Poor Responders were defined as patients presenting with clinical (relapses and/or increased disability) and/or MRI activity despite IFN therapy while GR included stable patients (no MRI or clinical activity for at least 2 years on IFN). While the majority of our enrolled patients were diagnosed as relapsing remitting multiple sclerosis (RRMS), 40% of our

Table 1
Patient demographics.

Demographics	Health controls	No therapy	Good Responders	Poor Responders
Sample size	25	24	25	25
Age, Mean ± SD	50 ± 9	52 ± 11	54 ± 7	51 ± 11
Females, n (%)	20 (80%)	22 (88%)	21 (84%)	20 (80%)
Caucasians, n (%)	25 (100%)	22 (88%)	24 (96%)	25 (100%)
Disease course:	–			
RR		19 (32%)	25 (42%)	15 (25%)
SP		5 (33%)	0 (0%)	10 (67%)
Disease duration, years	–	16.9 ± 11.5	18 ± 8.8	18.1 ± 11.1
EDSS, median (IQR)	–	3.5 (1.6)	2.3 (0.8)	4.4 (2.1)

Poor Responders were secondary progressive multiple sclerosis (SPMS) patients who continued to receive IFNβ1a due to ongoing relapses (Lublin and Reingold, 1996). Our untreated MS patient population was 80% RRMS, 20% SPMS (Table 1). All patients were evaluated clinically within one month of their study participation and assessed using the Expanded Disability Status Scale (EDSS) to quantify disability. EDSS scores can range from 0.0–10.0 with ambulatory MS patient scores 1.0–4.5 and scores 5.0–6.5 indicating degrees of impaired ambulation. Patients with scores ≥7 are not ambulatory.

The 50 subjects on IFNβ1a therapy had blood drawn in EDTA tubes immediately prior to an Avonex injection and again 48 h later. Healthy controls and patients not receiving IFNβ1a had blood drawn at 0 and 48 h. Samples were coded so that their diagnosis, treatment and study group remained unknown until data acquisition was complete.

2.3. Sample preparation

Whole blood samples, collected in EDTA vacutainers, were separated over Ficoll (Ficoll-Paque PLUS, GE Healthcare) and recovered leukocytes were lysed for total RNA (miRvana kit, Life Technologies, Grand Island, NY) or total protein (RIPA lysis buffer [Sigma, St. Louis, MO] with protease and phosphatase inhibitors [Thermo Scientific, Rockford, IL]). Plasma samples were also preserved.

2.4. Western blotting

Discontinuous SDS-PAGE (12/7/4%) gels were loaded with 40 μg of total protein lysates, run and transferred to PVDF membranes by standard methods. Westerns were probed with antibodies to Dicer (Cell Signaling Technology, Danvers, MA), βactin (Abcam, Cambridge, MA) and goat anti-rabbit-HRP (Promega, Madison, WI) and visualized on a BioRad ChemiDoc XRS CCR camera with FemtoGlow substrate (Michigan Diagnostics, Royal Oak, MI). Quantity One software (BioRad, Hercules, CA) was used to normalize the quantified Dicer and actin bands. Our data are presented as actin normalized Dicer protein levels (Dicer/actin protein level). A healthy control separate from our study population donated a larger volume of blood which provided a common protein sample included in every blot for further normalization.

2.5. RNA analyses

DICER1 and GAPDH mRNA were assayed by real time RT-qPCR (primer sequences can be found in Wiesen and Tomasi, 2009). Reverse transcription used Superscript II (Life Technologies) and real time PCR used Sybr Green master mix (Roche Diagnostics, Indianapolis, IN). All assays were carried out on an ABI 7900 HT (Life Technologies) and analyzed with SDS software. Relative expression levels were determined by the ΔΔCt method (Magner et al., 2000). GAPDH was used as a reference gene and both intra- and inter-assay CV (%) were <15%. In addition, ACTB, B2M, STAT1, MxA and TRAIL mRNA absolute expression levels were determined by real time RT-qPCR standard curve method