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MALT1 induced immune response is governed by miR-2909 RNomics

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ABSTRACT

The paracaspase mucosa-associated lymphoid tissue 1 (MALT1) has been widely recognized to play crucial role in lymphocyte activation, development and the generation of lymphomas through the modulation of innate and adaptive immune responses. Our results reported here provide evidence for the first time to support the view that MALT1 exerts its effect upon immune response involving genes coding for retinoic acid-inducible gene 1 (RIG1); interferon-β (IFN-β); apo-lipoprotein B mRNA-editing, enzyme-catalytic, polypeptide-like 3G (APOBEC3G); IFN-γ; chemokine (C–C motif) ligand 5 (CCL5) and interleukin-17 (IL-17) through the initiation of cellular miR-2909 RNomics. This ensures sustained expression of specificity protein 1 (SP1)-dependent regulation of genes that in-turn governs MALT1 induced immune response. Based upon these results, a mechanistic-pathway is proposed that links the epigenomic-interplay between MALT1 and miR-2909.

1. Introduction

The orchestrated cross-talk between coding RNAs and regulatory non-coding RNAs, within human genome, has provided a compelling evidence for the existence of flexible programming of T-cell effector functions depending upon time and space of its activation thereby ensuring functional plasticity within CD4 (+) T-cell subsets comprising of T-helper (Th1), Th2, Th17 and regulatory T-cells (Steward-Tharp et al., 2010). This flexible programming of T-cell repertoire has been widely recognized to be governed by the nuclear factor κB (NFκB) and Kruppel-like transcriptional factors especially KLF4 (Cao et al., 2010). NFκB has the ability to induce expression of IL-6 (Libermann and Baltimore, 1990) which is known to regulate the balance between regulatory T-cells (Treg) and Th17 cells (Kimura and Kishimoto, 2010). KLF4 has been shown to play crucial role in the development of IL-17 producing CD4 (+) T-cells (Cao et al., 2010; Lebson et al., 2010). It is pertinent to note that Apoptosis antagonizing transcription factor (AATF)-genome encoded novel miR-2909 not only has the inherent capacity to regulate large number of genes involved in immunity and cancer (Fig. 1) but also NFκB-dependent regulation of genes coding for KLF4; SP1; cylindromatosis (CYLD); APOBEC3G; IFN-β and IFN-γ was shown to be mediated through NFκB induced miR-2909 RNomics (Malik et al., 2014). Recently, MALT1 has emerged as a critical regulator of CYLD activity responsible for the modulation of NFκB and RIG1 dependent pathways (Berger et al., 2013; McAllister-Lucas et al., 2011; Thome et al., 2010). MALT1 has been shown to be essential for the development of encephalitogenic Th17 cells (Brüstle et al., 2012). MALT1 deficient T-cells have been found unable to induce NFκB and secrete IL-2 in response to TCR stimulation (Fontán and Melnick, 2013; Fontan and Melnick, 2012). Keeping in view the above–mentioned findings, the present study was undertaken to explore the following three specific issues: (a) Does MALT1 possess the capacity to regulate miR-2909 expression within normal human peripheral blood mononuclear cells (PBMCs)? (b) If yes, can MALT1 induce T-cell flexible immune response through the regulation of miR-2909 RNomics? (c) What mechanistic pathway connects MALT1 with miR-2909 RNomics regulated immune response?

2. Materials and methods

2.1. Bioinformatics analysis

Promoter sequences for different genes were obtained from Mammalian Promoter sequence database (http://rulai.cshl.edu/CSHLmpd2/). Putative binding sites for various transcription factors

Abbreviations: MALT1, paracaspase mucosa-associated lymphoid tissue 1; RIG1, retinoic acid-inducible gene 1; IFN-β, interferon-β; APOBEC3G, apo-lipoprotein B mRNA-editing, enzyme-catalytic, polypeptide-like 3G; CCL5, chemokine (C–C motif) ligand 5; IL-17, interleukin-17; NFκB, nuclear factor κB; KLF4, Kruppel-like factor 4; AATF, apoptosis antagonizing transcription factor; CYLD, cylindromatosis; TGF-β, transforming growth factor-β; RORγt, retinoid-related orphan receptor gamma t.

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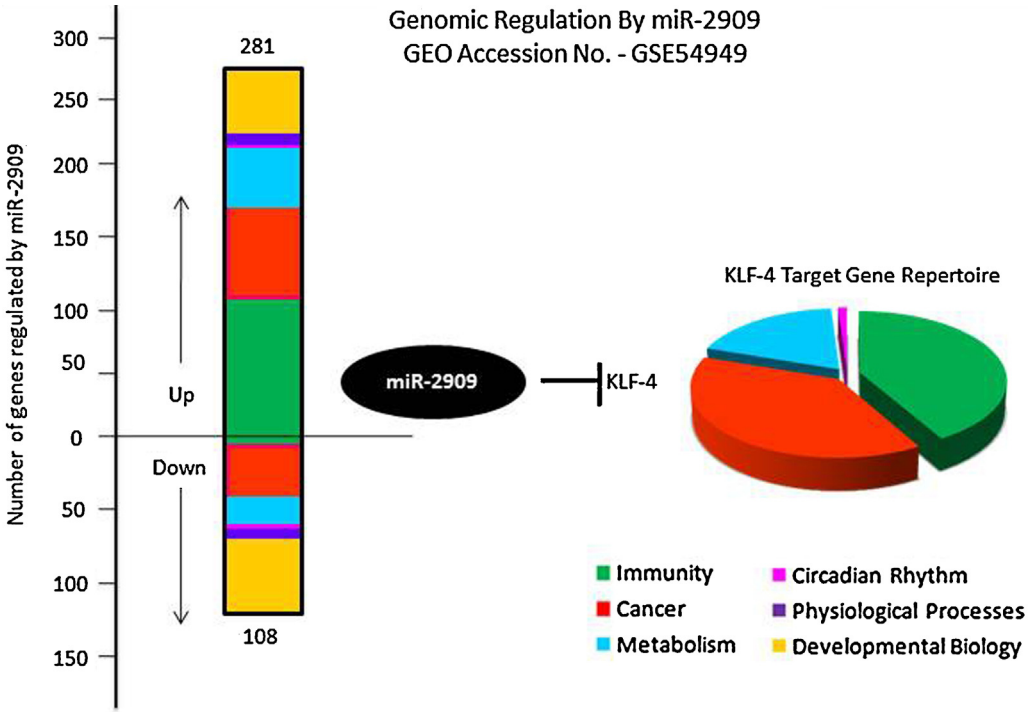

Fig. 1. Functional RNomics of miR-2909 involving KLF4 in HELA cells (NCBI; GEO accession no. —GSE54949).

were retrieved from JASPAR database (http://jaspar.cgb.ki.se/) and TFSEARCH (http://www.cbrc.jp/research/db/TFSEARCH.html) at a default threshold score of 85.0. The 5′ and 3′ UTR sequences of various genes were extracted from NCBI (http://www.ncbi.nlm.nih.gov/) and the presence of putative target sites of miR-2909 was depicted using RNA Hybrid tool (http://bibiserv.techfak.uni-bielefeld.de/rnahybrid/).

2.2. Cellular model employed

Human normal peripheral blood mononuclear cells (PBMCs) were obtained from 25 normal healthy volunteers (with their prior informed consent), who were fasting for 12 h or had abstained from any medication for 2 weeks before blood donation. Blood was drawn through venipuncture into heparinized tubes and PBMCs were isolated using density gradient centrifugation method. Briefly, 5 ml of heparinized blood was gently layered onto 4 ml of Histopaque (Sigma solution containing poly-sucrose and sodium diatrizoate, adjusted to a density of 1.077 ± 0.001 g/ml) and centrifuged at $400 \times g$ in swinging bucket rotors for 30 min at room temperature. After centrifugation, the layer of PBMCs was recovered & these cells were subsequently seeded in 24 well culture plates at an initial density of about 0.5×10^5 cells per well in RPMI-1640 medium containing 10% FCS (Fetal Calf Serum) at 37 °C in 5% CO_2. The cells were counted in hemocytometer chamber and viability was determined by Trypan Blue dye exclusion method. Further, the study conforms to the principles outlined in the declaration of Helsinki (Williams, 2008).

2.3. Cell transfection experiments

p3F-Strep-mMALT1 obtained from "Addgene plasmid 33315" (Stempin et al., 2011) was transfected into PBMCs using ESCORT transfection reagent (Sigma) to overexpress MALT1 protein. siRNA's against conserved sequence of MALT1 mRNA (Sigma) was used to knock-down the expression of MALT1. miR-2909 expression was modulated using miR-2909 expression plasmid (SBI) and mercury LNA™ miR-2909 inhibitor (EXIQON). PMXs-hKLF4 (Plath) obtained from "Addgene plasmid 17967" was used to over-express KLF4 gene. Transfection efficiency of 70–75% was measured by transfecting a control GFP vector into normal PBMCs maintained under similar conditions as compared to their corresponding controls. The control PBMCs were simultaneously transfected with control vectors and or scrambled RNA sequences (50 nM) using the same transfection reagent under similar conditions in order to avoid any artifacts induced by the transfection reagent. The sequences corresponding to the coding region of APOBEC3G were amplified using gene specific primers and cloned into pEF6V5His TOPO expression vector (Invitrogen). In each experiment, the cells were maintained in vitro in RPMI-1640 culture medium and incubated up to 48–72 h at 37 °C in humidified 5% CO_2 atmosphere. At the end of incubation period, the cells harvested from each well were subjected to RNA and protein isolation using standard methods (Sharma et al., 2013). SP1 transcription factor binding site containing sequence on MALT1 promoter was amplified using PCR and subsequently the amplicon was cloned into pBlue TOPO reporter vector (Invitrogen). The vector was transfected into normal PBMCs. After 24 h, these cells were transfected with KLF4 expression plasmid and incubated for 48 h at 37 °C under 5% CO_2-atmosphere. At the end of this incubation period, the cells from each well were processed for the measurement of β-galactosidase activity by specific assay kit (Invitrogen).

2.4. Gene expression analysis

The isolated RNA from each culture-well was subjected to expression analysis of genes coding for miR-2909, IL6, CCL5, KLF4, MALT1, IFN-β, IFN-γ, RIG1 and APOBEC3G using gene-specific primers (Table 1) and Real-Time PCR method. U6 and GAPDH (Table 1) were used as invariant controls for the expression analysis of miR-2909 and other genes, respectively. The isolated proteins from each culture-well were subjected to SDS-PAGE followed by western Immuno-detection using specific antibodies against MALT1, KLF4, SP1, CCL5, IL-17, IL-6, APOBEC3G and β actin

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