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High glucose-induced human cellular immune response is governed by miR-2909 RNomics

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ABSTRACT

Regulation of NFkB family member RelA translocation by tumour suppressor genes encoding p53 and KLF4, has been widely recognized as the critical for human peripheral blood mononuclear cells (PBMCs) to meet their energy requirement for tailoring their immune response against any perceived threat. Our study was addressed to understand as to how human PBMCs respond to high glucose threat in terms of their genomics-directed immune response. The results of such a study revealed for the first time that NFkB induced miR-2909 RNomics is crucial for the regulation of RelA translocation within human PBMCs exposed to high glucose thereby enabling these epigenetically programmed cells to tailor immune response involving genes coding for CCL5; IFN-γ and IL-17. Based upon these results an attempt was also made to propose a mechanistic pathway that links high glucose induced cellular miR-2909 RNomics with the genes involved in energy metabolism and immune response.

1. Introduction

Aerobic-glycolysis is not only the unique feature of cancer cells but also of the normal immune/stem cells that use this phenomenon to produce intermediary metabolites of glycolysis as substrates for growth [1,2]. The cellular cyclic-transition from aerobic respiration to glycolysis would have been impossible without the existence of master-regulatory genes coding for p53 and NFkB family member RelA because p53-dependent genomics promotes aerobic-respiration leading to cellular differentiation [3,4] whereas RelA-dependent genomics initiates aerobic-glycolysis reverting fully differentiated cells into more primitive de-differentiated form [5]. Recently, a novel emerging role of p53 in curtailing the stem cell expansion has unfolded the mechanisms through which p53 restricts self-renewing divisions and reprogramming of somatic cells into stem cells [6]. Importantly, KLF4 was shown to physically interact with p53, resulting in the synergistic activation of p21 promoter thereby acting as a critical mediator of p53-induced growth arrest [7]. It is pertinent to note that the polycomb group protein Bmi-1 has been shown to support stem cells renewal by ensuring reduced p53 protein half-life [8] whereas KLF4 restricts the transcriptional expression of Bmi-1 [9]. Further the stimulating effect of Bmi-1 upon NFkB activation [10] is reversed by both p53 as well as KLF4 through the inhibition of IKK [3,11]. The tumour suppressor p53 not only restricts cancer cell addiction for sugar through repression of glucose transporters 1 and 4 (GLUT 1 and GLUT 4) but also inhibits glucose-induced cell proliferation through the activation of NFkB [3]. Interestingly, PPAR-γ also has the capacity to inhibit NFkB activation [12] as well as increase in the expression of genes coding for KLF4 [13] and GLUT1 but not GLUT4 [14]. Cellular PPAR-γ mediated transcriptional activation has been shown to be suppressed by PER-3 [15]. Apart from the role of these genes, coding for KLF4, p53, NFkB and PPAR-γ, in the cellular metabolism and differentiation, they also play crucial role in programming the cellular immune response. KLF-4 has been found to play crucial role in the development of IL-17 producing $CD4^+$ T-cells independently of RORγt [16] whereas p53 was shown to induce type-1 interferons and regulatory T-cells [17,18] as well as to inhibit translational expression of IFN-γ [19,20]. PPAR-γ was shown to restrict $CD4^+$ T-cell differentiation to Th1 and Th17 cell subsets and promote Th2 differentiation [21] as well as favour phenotype-switch from Th17 into Treg $CD4^+$ T cells [22]. At this stage, it is pertinent to note that NFkB was shown to regulate the expression of a novel microRNA (designated as miR-2909) encoded by human cellular AATF-genome [11,23]. This miR-2909 was shown to regulate a large number of genes involved in the immunity and cancer (Fig. 1) through its capacity to suppress KLF4 translational expression [11,23,24]. Keeping in view the fact that orchestrated cross-talk between coding RNAs and regulatory

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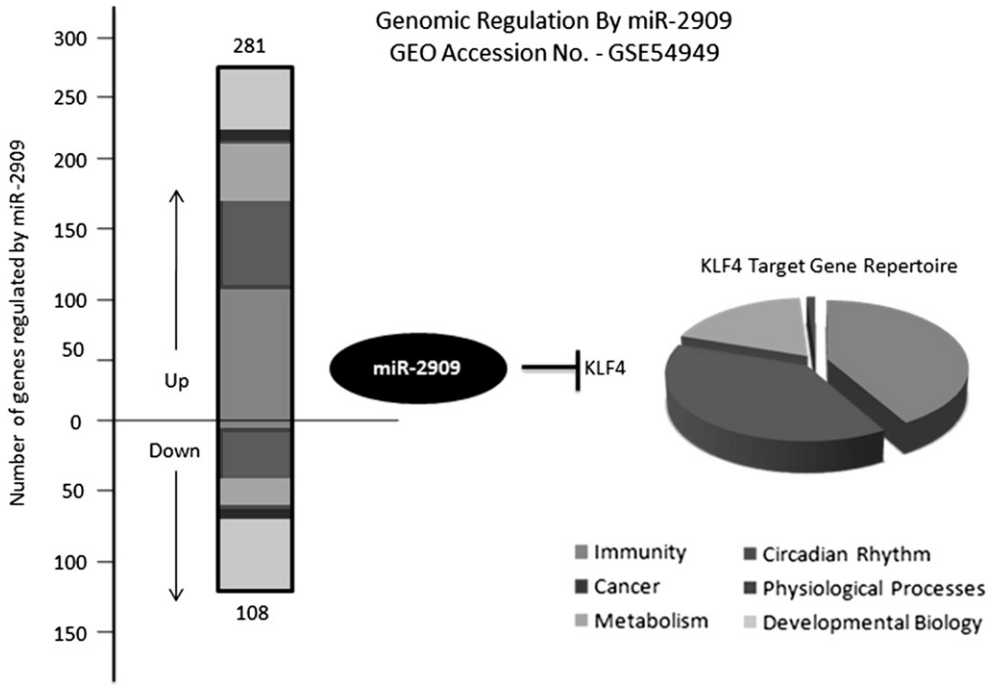

Fig. 1. Functional miR-2909 RNomics in HeLa cells: miR-2909 RNomics governed by regulatory-link between miR-2909 and KLF4-dependent genes involved in immunity, cancer, metabolism etc. (NCBI: Geo Accession No. GSE54949).

miRNAs (within human genome) has provided compelling evidence for the existence of flexible programming of immune cells depending upon time and space of their activation, the present study was addressed to understand as to how human peripheral blood mononuclear cells (PBMCs) exposed in vitro to high-glucose respond to this threat in terms of influencing their miR-2909 RNomics involving genes that link energy metabolism with immune response.

2. Methods

2.1. Cellular model and invitro culture design

Human PBMCs were isolated from healthy volunteers, who were fasting for 12 h and had abstained from any medication for 2 weeks before blood donation, using Ficoll-Hypaque density gradient method as reported earlier [11]. Subsequently these cells were exposed to RPMI-1640 culture medium enriched with 25 mM glucose and incubated up to 72 h at 37 °C in 5% CO_2 atmosphere.

2.2. High glucose-induced cellular RelA translocation

The cells from each culture well, at different incubation periods ranging from 0–72 h, were processed to prepare cytosolic and nuclear-protein extracts reported by us earlier [11]. These protein samples were subsequently subjected to SDS page followed by Western blotting and immunodetection using specific antibodies against phospho IkBα, RelA and Histone H3 (used as invariant control).

2.3. High glucose programmed cellular genomic expression

At the end of specified incubation periods, the cells from each well were processed for total cellular as well as small non-coding RNA extraction using miReasy mini kit (Qiagen). The extracted RNA was reverse transcribed using miScript reverse transcription kit (Qiagen). Differential expression of genes coding for miR-2909; PER3; NANOG; GLUT1; PPAR-γ; Bmi-1; IFNγ; Cyclin 'E'; C-myc; p53 was studied with real time PCR using gene specific primers (Table 1). β_2M and U6 were used as invariant controls for expression studies of various genes and miR-2909 respectively. Total cellular protein was isolated using standard Lammelli's method [11,24]. The isolated proteins from each culture well were subjected to Western-blotting followed by immunodetection using specific antibodies against Cyclin 'D', KLF4, IL-17, CCL5, p53 and Histone H3 (used as an invariant control). Each band on the immunoblot was scanned densitometrically using Scion Image Analysis software, and the results were expressed as intensity ratio of target protein to histone protein taken as AU (arbitrary unit).

2.4. KLF4-dependent genomics

Normal human PBMCs were transfected with either KLF4 expression plasmid (Addgene plasmid 17967) or control plasmid with scrambled-insert using escort transfection reagent (Sigma) and incubated for 48 h in nutrient medium at 37 °C in 5% CO_2 atmosphere. At the end of incubation period cells from each well were processed for RNA isolation and cDNA preparation as described earlier. The expression of various

Table 1
Primer sequences.

Gene	Forward primer (5′ → 3′)	Reverse primer (5′ → 3′)
NANOG	5′GTCTTCTGCTGAGATGCCTCACA3′	5′CTTCTGCGTCACACCATTGCTAT3′
Bmi-1	5′TCTGCAGCTCGCTTCAAGAT3′	5′AGTGGTCTGGTCTTGTGAAC3′
GLUT1	5′TGAGCATCGTGGCCATCTTT3′	5′CCGGAAGCGATCTCATCGAA3′
PPAR-γ	5′GCATTATGAGACATCCCC3′	5′GCGATTCCTTCACTGATAAC3′
IFN-γ	5′GTTTGGGTTCTCTTGGCTGTT3′	5′CTCCTTTTTCGCTTCCCTGTTTT3′
CCL5	5′CGTGCCCACATCAAGGAGTA3′	5′CTTCTCTGGGTTGGCACACA3′
p53	5′GAAGACCCAGGTCCAGATGA3′	5′CTGCCCTGGTAGGTTTTCTG3′
Cyclin E	5′GACATACTTAAGGGATCAGC3′	5′GGGGACTTAAACGCCACTTA3′
C-myc	5′CGGAACTCTTGTGCGTAAGG3′	5′GGATTGAAATTCTGTGTAACTGC3′
β2M	5′GAATTGCTATGTGTCTGGGT3′	5′CATCTTCAAACCTCCATGATG3′