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The impact of microRNA expression on cellular proliferation

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Abstract

A s an important class of non-coding regulatory RNA s, microRNA s (miRNAs) play a key role in a range of biological processes. These molecules serve as post-transcriptional regulators of gene expression and their regulatory activity has been implicated in disease pathophysiology and pharmacological traits. We sought to investigate the impact of miRNAs on cellular proliferation to gain insight into the molecular basis of complex traits that depend on cellular growth, including, most prominently, cancer. We examined the relationship between miRNA expression and intrinsic cellular growth (iGrowth) in the HapMap lymphoblastoid cell lines derived from individuals of different ethnic backgrounds. We found a substantial enrichment for miRNAs (53 miRNAs, FDR < 0.05) correlated with cellular proliferation in pooled CEU (Caucasian of northern and western European descent) and YRI (individuals from Ibadan, Nigeria) samples. Specifically, 119 miRNAs (59 %) were significantly correlated with iGrowth in YRI; of these miRNAs, 18 were correlated with iGrowth in CEU. To gain further insight into the effect of miRNAs on cellular

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proliferation in cancer, we showed that over-expression of miR-22, one of the top iGrowth-associated miRNAs, leads to growth inhibition in an ovarian cancer cell line (SKOV3). Furthermore, over-expression of miR-22 down-regulates the expression of its target genes (*MXI1* and *SLC25A37*) in this ovarian cancer cell line, highlighting an miRNA-mediated regulatory network potentially important for cellular proliferation. Importantly, our study identified miRNAs that can be used as molecular targets in cancer therapy.

Introduction

Cellular growth is fundamental to cell biology. Thus, a greater understanding of contributing factors to this trait can improve our understanding of biological phenomena, and as a translational application, enable the development of therapeutic strategies against pathological conditions (e.g., cancers and aging).

It has been shown that cellular growth is a heritable trait and genetic variation is likely to contribute to phenotypic variability (Stark et al. 2010). Many genes are known to affect cellular growth, including those controlling cell cycles (Schafer 1998; Whitfield et al. 2006). More recently, micro-RNA s (miRNAs), a class of small non-coding regulatory RNA s, have been implicated in a wide range of biological processes (Bartel 2009; Suzuki and Miyazono 2011). They also play a key role in tumorigenesis and tumor suppression in cancers (Hwang and Mendell 2006). Thus, we hypothesized that miRNAs might be the important regulators of cellular proliferation and set out to perform a genome-wide study of the impact of miRNA expression on cellular growth.

For genome-wide discovery, we utilized a set of the International HapMap lymphoblastoid cell lines (LCLs). These HapMap LCLs have extensive publicly available genomic information (Frazer et al. 2007; The 1000 Genomes Project Consortium 2010), as well as other omics data (Duan et al. 2008; Stranger et al. 2007; Pickrell et al. 2010). More recently, we have generated whole-genome miRNA expression data on a subset of the HapMap samples (Gamazon et al. 2012) (GEO accession number GSE34406). The depth of this publicly available information makes HapMap LCLs an unparalleled resource to conduct "omics" research in human complex traits. In this study, we performed genome-wide analysis using miRNA expression, mRNA expression and cellular growth as a phenotype. We then evaluated whether the miRNA /gene(s) identified in the HapMap cell lines also affect cellular growth in an ovarian cancer cell line.

Results

Intrinsic cellular growth (iGrowth) associated miRNA expression

We have previously generated an intrinsic cellular growth (iGrowth) phenotype for over 500 HapMap LCLs using a mixed effect model averaging (MEM) method to control for various environmental conditions [e.g., passage of cells, time of experiment, different experimental conditions (batch of serum/media)] (Im et al. 2012).

To detect significant correlations between iGrowth and genome-wide miRNA expression, we performed a pooled analysis of the combined CEU (Centre d'Etude du Polymorphisme

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Humain (CEPH) people from Utah, USA) and YRI (Yoruba people from Ibadan, Nigeria) samples (see “Materials and methods”). As a result, we found 53 miRNAs that were significantly correlated with iGrowth (false discovery rate [FDR] <0.05, listed in Table 1). Among them, 14 (nearly 7 %) showed FDR < 0.001.

In addition, we performed linear regression analysis between iGrowth and genome-wide miRNA expression in the YRI and CEU samples separately. A Q–Q plot shows a substantial enrichment for significant associations with iGrowth among the miRNAs in the YRI samples (Fig. 1). Indeed, 119 miRNAs were found to be significantly correlated with iGrowth in these samples (FDR < 0.05). Eighteen of these 119 miRNAs were also correlated with iGrowth in CEU samples (Supplementary Table 1). Among 18 miRNAs, 15 miRNAs were also significantly correlated with iGrowth in the pooled analysis. Interestingly, eight (miR-185*, miR-18b, miR-365, miR-600, miR-768-3p, miR-768-5p, miR-939 and miRPlus_42521) of the 18 miRNAs with significant correlation with iGrowth in both populations are differentially expressed between the two panels ($p < 0.05$). An example is shown in Fig. 2a. Here, miR-768-5p is correlated with iGrowth in both CEU and YRI samples ($p = 0.0024$ and $p < 0.0001$ in CEU and YRI, respectively); however, higher miR-768-5p expression was observed in YRI relative to CEU ($p < 0.05$). Furthermore, we identified other patterns of miRNA-iGrowth relationship. For example, miR-22 expression was found to be significantly and negatively correlated with iGrowth in YRI samples ($p < 0.0001$) but showed only modest correlation with iGrowth in CEU ($p = 0.067$) (Fig. 2b).

Biological targets of miRNA involved in cellular growth

To explore the underlying mechanism for the observed miRNA and cellular growth associations, we further examined the relationships between iGrowth-associated mRNA s (reported previously in Im et al. 2012) and iGrowth-associated miRNAs identified in the pooled analysis. Of the 53 miRNAs associated with iGrowth in the pooled CEU and YRI samples (FDR < 0.05), 22 miRNAs are negatively correlated with one of 105 growth-associated mRNA s ($p < 0.0001$, FDR < 0.05) in CEU and 42 miRNAs are negatively correlated with one of 211 growth-associated mRNA s ($p < 0.0001$, FDR < 0.05) in YRI. These iGrowth-associated mRNA s were evaluated using Database for Annotation, Visualization and Integrated Discovery (DAV ID) for gene ontology (GO) annotations (Huang et al. 2009). GO analysis yielded various biological processes, molecular functioning and cellular component pathways listed in Supplementary Table 2 ($p < 0.05$). Not surprisingly, among them were the regulation of cellular proliferation and regulation of cellular growth pathways ($p = 0.0029$ and $p = 0.0035$, respectively).

Among the top iGrowth-correlated miRNAs, miR-22 was negatively correlated with the expression levels of 39 genes in the YRI samples ($p < 0.0001$, FDR < 0.05, Fig. 3). Notably, the expression of miR-22 and all 39 genes was correlated with iGrowth in these samples. Two gene/miR-22 relationships [Max-interacting protein (*MXI1*) and Solute carrier family 25, member 37 (*SLC25A37*)] are also supported by the miRbase miRanda prediction algorithm.

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Evaluation of the function of miR-22 in an ovarian cancer cell line

Given that miR-22 was among the very top iGrowth-correlated miRNAs (i.e., 5th) and was negatively correlated with 39 gene expression phenotypes, we performed additional studies on the function of this miRNA in cancer. We conducted miR-22 over-expression experiment in an ovarian cancer cell line (SKOV3). As expected, the transfection of miR-22 mimic (over-expression) resulted in significantly increased expression of miR-22 (compared to control, *t* test $p = 2.4 \times 10^{-05}$, Fig. 4a) at 24 h post-transfection. Subsequently, we observed a significant inhibition in cellular growth, measured by cellular AT P levels (see "Materials and methods"), 72 h after over-expressing miR-22 when compared to that of scramble control ($p = 0.0014$, Fig. 4b). Over-expression of miR-22 also resulted in significant decrease in expression levels of *MXI1* and *SLC25A37* (negatively correlated and predicted by miRbase miRanda prediction algorithm) in this cancer cell line ($p = 0.003$ and $p = 0.001$, respectively, Fig. 4c).

Discussion

Through a genome-wide analysis, we found a set of miRNAs whose expression correlated with intrinsic cellular growth in LCLs. Furthermore, as a proof of concept, we demonstrated that over-expression of one of the top cell growth-associated miRNAs can significantly inhibit the proliferation of ovarian cancer cells.

From the pooled analysis and the single-population analyses, we identified 15 miRNAs whose expression levels are correlated with iGrowth in both CEU and YRI samples. Eight of these miRNAs have been previously implicated in cellular proliferation and cancer-related pathways. For example, miR-210 was found to be over-expressed in late stages of lung cancer (Puisségur et al. 2011), and disturbed mitotic progression (He et al. 2012). miR-148b was previously reported to be a tumor suppressor in gastric cancer (Song et al. 2011a), colorectal cancer (Song et al. 2011b), oral squamous cell carcinoma (Yu et al. 2009), as was miR-185 (Imam et al. 2010). miR-365 regulates lung cancer through translational repression of *TTF1* (Thyroid transcription factor 1) (Qi et al. 2012). When down-regulated, miR-193b was shown to contribute to the development of T-cell lymphoblastic lymphoma (González-Gugel et al. 2013) and tumor progression in human breast cancer (Li et al. 2009). Let-7d was reported to inhibit neural stem cell proliferation in mammalian brains (Zhao et al. 2013) and involved in regulation of *PBX3* (pre-leukemia transcription factor 3) expression in prostate cancer cell lines (Ramberg et al. 2011). miR-34a was involved in inhibition of prostate cancer cell growth (Kashat et al. 2012). miR-34a was also regarded as tumor suppressor in hepatocellular carcinoma (Dang et al. 2013) and regulator of growth factor signaling (Lal et al. 2011). Finally, miR-18b was found to be up-regulated in four out of five breast cancer cell lines, and ectopic inhibition of miR-18b suppressed the migration of two breast cancer cell models in vitro (Fonseca-Sanchéz et al. 2013). Our observation on the effect of the remaining 7 miRNAs on cellular proliferation is, to our knowledge, a novel finding. We note that, for each of the miRNAs, expression is correlated with cellular proliferation in two independent sample sets.

Among the miRNAs with significant correlation with cellular proliferation in the pooled analysis, miR-22 has been found to play a key role in a number of biological pathways. For

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example, miR-22 was reported to directly target estrogen alpha (Pandey and Picard 2009; Xiong et al. 2010a), *MYCBP* (Xiong et al. 2010b), *PTEN* (Bar and Dikstein 2010; Liu et al. 2010) and *HDAC4* (Huang et al. 2013). It also affects MyC (Xiong et al. 2010b), p53-dependent apoptosis (Tsuchiya et al. 2011), NFkB (Takata et al. 2011), Wnt signaling (Kaur et al. 2011), AKT (Bar and Dikstein 2010), and PKC/ERK (Ting et al. 2010) pathways. Because of its extensive influence on these pathways, miR-22 has been reported to play a role in cellular senescence induction (Xu et al. 2011), migration (Liu et al. 2010; Xu et al. 2011; Li et al. 2010), angiogenic (Yamakuchi et al. 2011) and cell cycle arrest (Ting et al. 2010) in various cancer cell lines. Moreover, miR-22 expression has been shown to be associated with radioresistance (Zheng et al. 2011). In an ovarian cancer cell line, miR-22 was previously identified as a potential metastasis inhibitor (Li et al. 2010). Previous studies have also showed that ectopic expression of miR-22 significantly inhibits cell proliferation and tumorigenicity in hepatocellular carcinoma (Zhang et al. 2010). Our study confirmed a similar effect of miR-22 on ovarian cancer cell proliferation, with over-expression of miR-22 resulting in growth inhibition.

miRNAs are known to regulate gene expression at the post-transcriptional and translational stage (Morris et al. 2004). Based on DAV ID gene ontology, functional enrichment analysis of the genes (negatively correlated to miRNA from pooled analysis) revealed their involvement in pathways regulating cellular proliferation and cellular growth. The analysis also revealed that they were mostly present in cytosol involved in protein binding. Among the relationships of iGrowth-associated miRNA and iGrowth- associated mRNA, we found that 39 genes were negatively correlated with miR-22, suggesting a potential miR-22 regulated gene network that may mediate the cellular growth phenotype. In an ovarian cancer cell line, we showed that overexpression of miR-22 resulted in decreased gene expression level for putative targets *MXI1* and *SLC25A37*, which may suggest a biological mechanism underlying the miR-22 and iGrowth correlation. *MXI1* forms a heterodimer with Max, similar to that of Myc-Max (Zervos et al. 1993), which is involved in controlling cell proliferation and tumorigenesis in different biological contexts (Hurlin and Dezfouli 2004). *SLC25A37* is a solute carrier that transports iron into the mitochondria of erythroid cells for the synthesis of heme and iron sulfur clusters (Chen et al. 2009). It has been reported that over-expression of *SLC25A37* causes cancer-related fatigue in patients with non-metastatic prostate cancer during external beam radiation therapy (Hsiao et al. 2013). It is important to note that although we showed that over-expression of miR-22 leads to decreased expression of *MXI1* and *SLC25A37*, we did not perform the same functional study on the expression of the remaining 37 (potential target) genes, some of which are supported by previous studies such as the reduction of tumor cell proliferation when Vascular Endothelial Growth Factor A (*VEGFA*) was targeted in mouse model with ovarian granulosa cell tumor (Tsoi et al. 2013). Additional studies of miR-22 and its target genes in cancers and other pathological contexts are warranted.

In summary, our genome-wide study identified and replicated several miRNAs with significant effect on cellular proliferation. We performed additional functional studies to confirm the effect of miR-22 on cellular growth in LCLs and in an ovarian cancer cell line.

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These miRNAs are great candidates for future investigations of growth-mediated phenotypes and may be potential targets for cancer therapeutics.

Materials and methods

Cell lines

EBV-transformed B-LCLs from the International HapMap consortium were purchased from the Coriell Institute for Medical Research (Camden, NJ, USA). Fifty-three unrelated CEU and 54 unrelated YRI samples were used for this study. These LCLs were maintained as suspension cultures in RPMI 1640 with supplements described previously (Huang et al. 2007). For functional studies, SKOV3, an ovarian cancer cell line, was procured from AT CC (Manassas, VA, USA) and grown as an adherent culture in McCoy's 5A medium with 10 % fetal bovine serum (Atlanta Biologicals, GA).

Intrinsic cellular growth rate and genomic, transcriptomic, miRNA expression information

Utilizing a mixed effects model averaging (MEM) method, our group has previously generated an intrinsic cellular growth (iGrowth) phenotype in over 500 HapMap LCLs (Im et al. 2012). This method allowed us to pool data from multiple replicated measurements over a 5-year time period and obtain an intrinsic cell growth phenotype after controlling for various environmental conditions [e.g., passage of cells, time of experiment, different experimental conditions (batch of serum/media)]. The iGrowth for the CEU and YRI samples was used in this study.

We performed genome-wide miRNA expression profiling, as previously reported (Gamazon et al. 2012), using the Exiqon miRCURY LNA arrays v.10.0 (Exiqon array) on 107 LCLs (53 CEU I and 54 YRI I). These data have been deposited into GEO (GSE34406). The baseline genome-wide gene expression data in CEU and YRI were quantified using the Affymetrix GeneChip Human Exon 1.0 ST array (Affymetrix exon array, GEO access #: GSE7761) (Zhang et al. 2008). In the present study, we evaluated the set of genes previously shown to be correlated with iGrowth (Im et al. 2012).

iGrowth-associated miRNA expression

Linear regression analysis was performed between 201 miRNAs that are reliably expressed in LCLs and iGrowth in the HapMap CEU and YRI samples jointly and separately using an R package (lm). In a pooled analysis using the combined CEU and YRI samples, we performed linear regression of miRNA expression against iGrowth with the ancestral group as covariate. For multiple testing adjustment, we used an FDR approach (Storey and Tibshirani 2003). FDR < 0.05 was used as the threshold for significance.

We performed permutation analysis to assess the enrichment of miRNAs significantly correlated with iGrowth. In this analysis, we permuted the iGrowth trait values ($n = 1000$ permuted datasets) while preserving the correlation structure of the miRNAs. Correlation analysis was performed on each permuted dataset and the distribution of p values for each permuted dataset was plotted in a Q–Q plot along with the observed distribution of p values.

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We have reported 2983 mRNA s that are correlated with iGrowth in these cell lines previously. In this study, to explore the potential mechanism underlying the observed miRNA and iGrowth relationships, we further examined the negative correlation between iGrowth-associated miRNAs and mRNA s; $p < 0.0001$ was used as a cutoff, which corresponds to FDR < 0.05. Gene ontology analysis was performed using the functional annotation tool in DAV ID Bioinformatics resources v6.7 (Huang et al. 2009).

Functional validation

For the functional validation of iGrowth-associated miRNA expression, miR-22 over-expression was performed in SKOV3, an ovarian cancer cell line. miR-22 mimic and scrambled control (AllStars negative control siRNA), purchased from Qiagen, were independently transfected into SKOV3 using DharmaFECT 1 (Thermo scientific) according to Thermoscientific DharmaFECT siRNA transfection protocol for 6 h. The cellular growth rate was measured using CellTiter-GLo luminescent cell viability assay (QIAGEN) at 0, 48 and 72 h post-transfection. Student *t* test was performed to compare cellular growth rate obtained at 72 h post-transfection between miR-22 over-expressed samples and control with scramble treatment. $p < 0.05$ was considered statistically significant. The over-expression was confirmed by performing quantitative polymerase chain reaction (qPCR) on 24 h post-miR-22 transfection samples. Further, the gene expression levels of *MXI1* and *SLC25A37* were quantified through qPCR 24 h post-transfection using TaqMan® assays (Hs00365651_m1 and Hs00249769_m1, respectively, Life Technologies).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

miRNA	microRNA
iGrowth	Intrinsic cellular growth
LCLs	Lymphoblastoid cell lines
CEU	Centre d’Etude du Polymorphisme Humain (CEPH) people from Utah, USA
YRI	Yoruba people from Ibadan, Nigeria
MEM	Mixed effects model averaging

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FDR	False discovery rate
DAVID	Database for Annotation, Visualization and Integrated Discovery
GO	Gene ontology
DOHH	Deoxyhypusine hydroxylase

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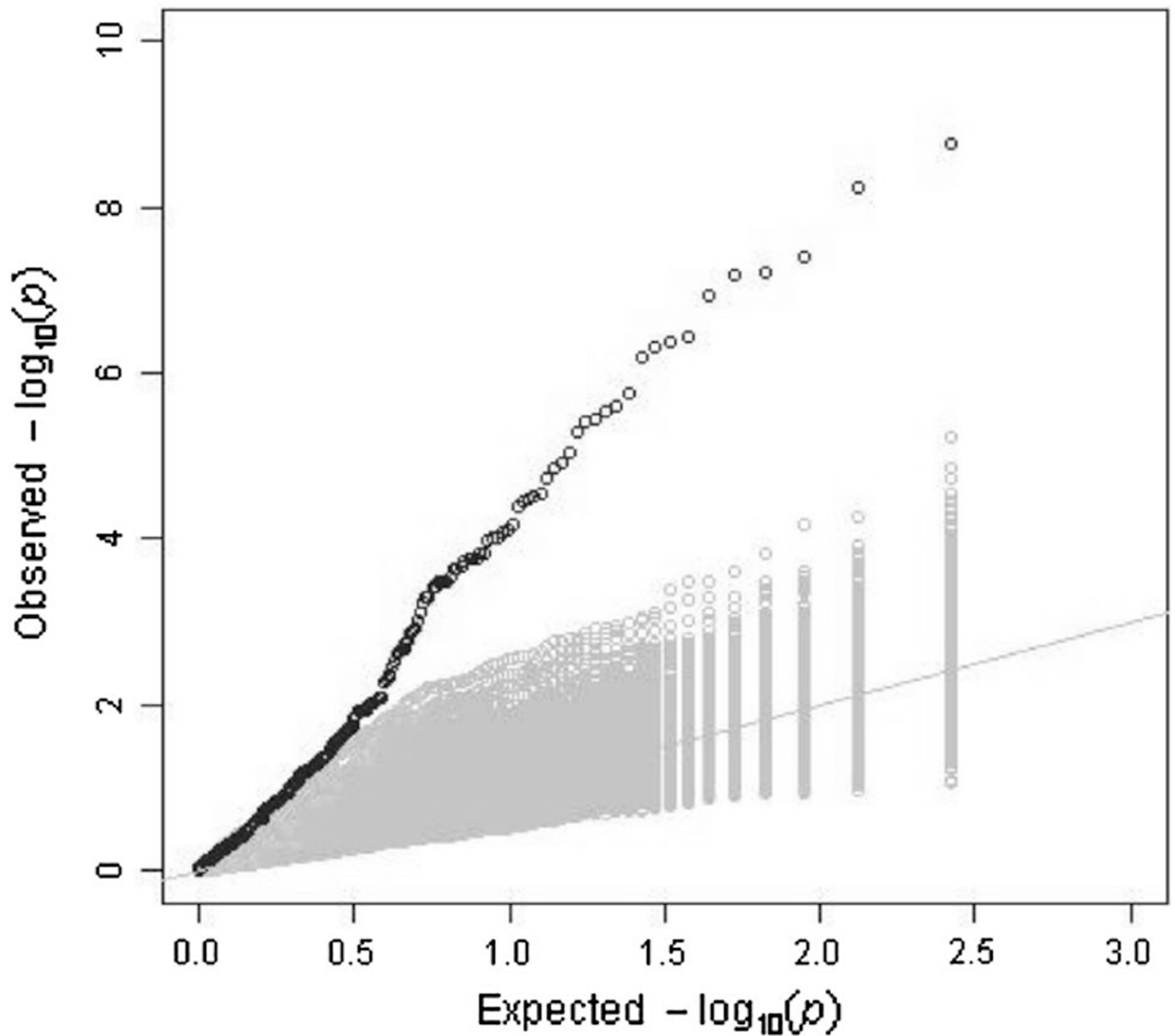

Fig. 1.

Q–Q plot of iGrowth-associated miRNAs in YRI samples. We observed an excess of significant correlations of miRNAs with iGrowth in YRI. The Q–Q plot, in black, shows the observed distribution of *p* values from the correlation between miRNA and iGrowth. We also generated permuted datasets ($n = 1000$) by shuffling the iGrowth phenotype while preserving the miRNA correlation structure. The Q–Q plots, in gray, show the distributions of *p* values from the correlation between miRNA and iGrowth in the permuted datasets

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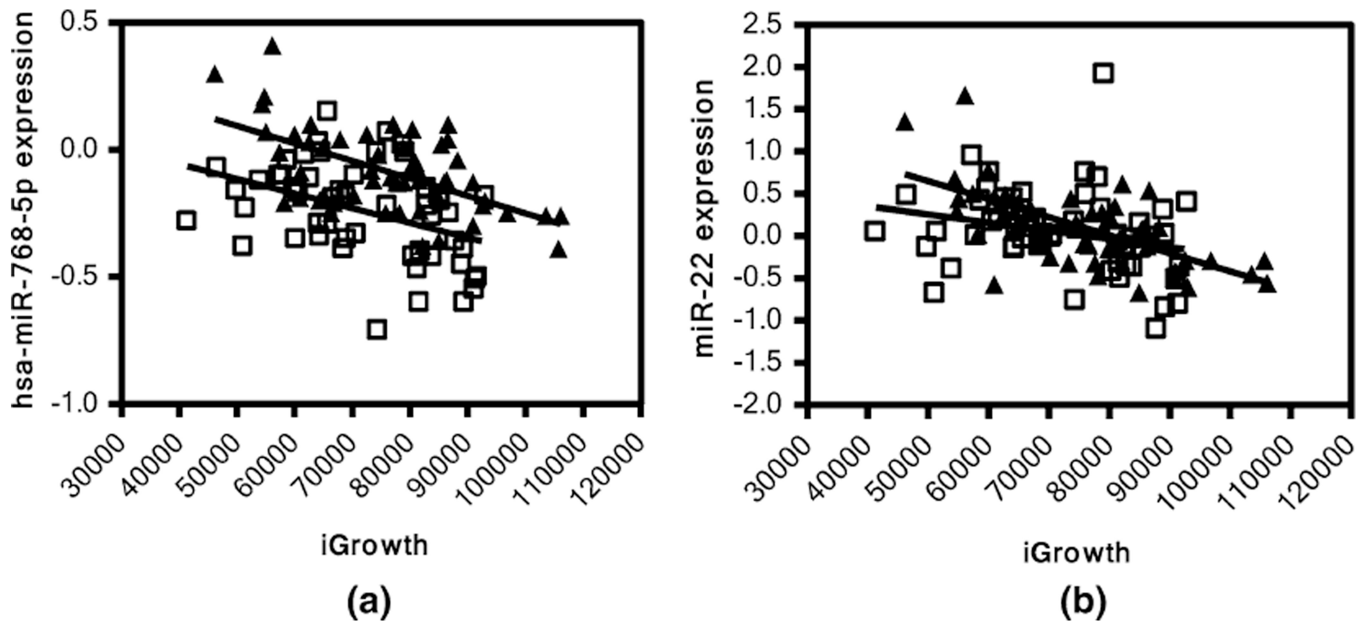

Fig. 2.

Pattern of correlation between miRNA and iGrowth in CEU and YRI. **a** miR-768-5p is significantly correlated with iGrowth in both CEU ($p = 0.0024$) and YRI ($p < 0.0001$) samples; **b** miR-22 is significantly correlated with iGrowth in YRI ($p < 0.0001$) but only modestly in CEU ($p = 0.0673$). *Triangle* represents YRI samples and *square* represents CEU samples

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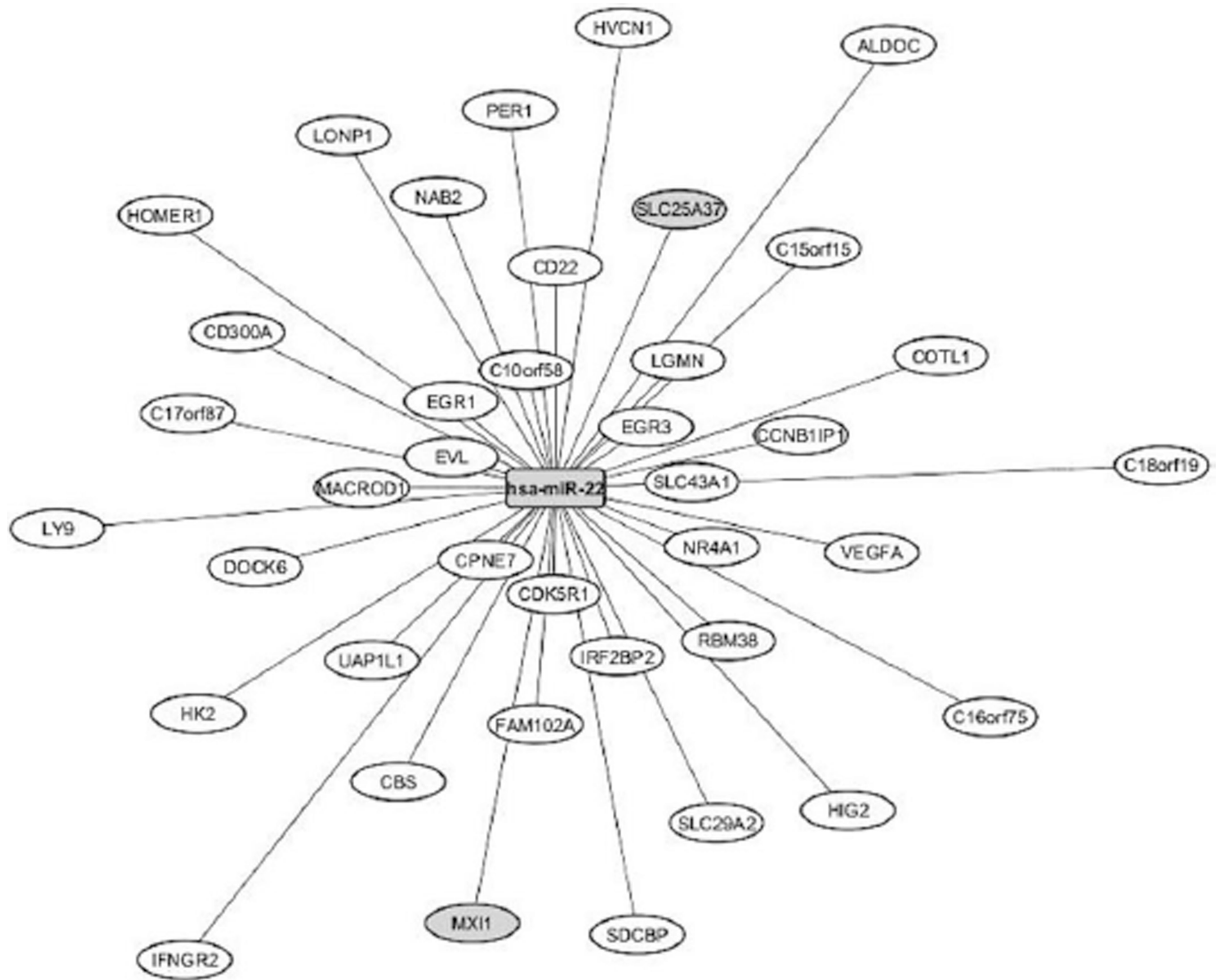

Fig. 3.

Negative expression correlation of miR-22 and 39 genes. The expression levels of all genes are correlated with iGrowth in YRI samples. miR-22 ranked 5th in association with iGrowth in pooled analysis of CEU and YRI ($p = 1.7 \times 10^{-6}$). The distance between the central node, miR-22, and the other nodes (comprising of the 39 genes) reflects the p value of the expression correlation between miRNA and gene: the shorter the distance, the more significant is the miR-gene expression correlation. The graph was generated using the cytoscape software

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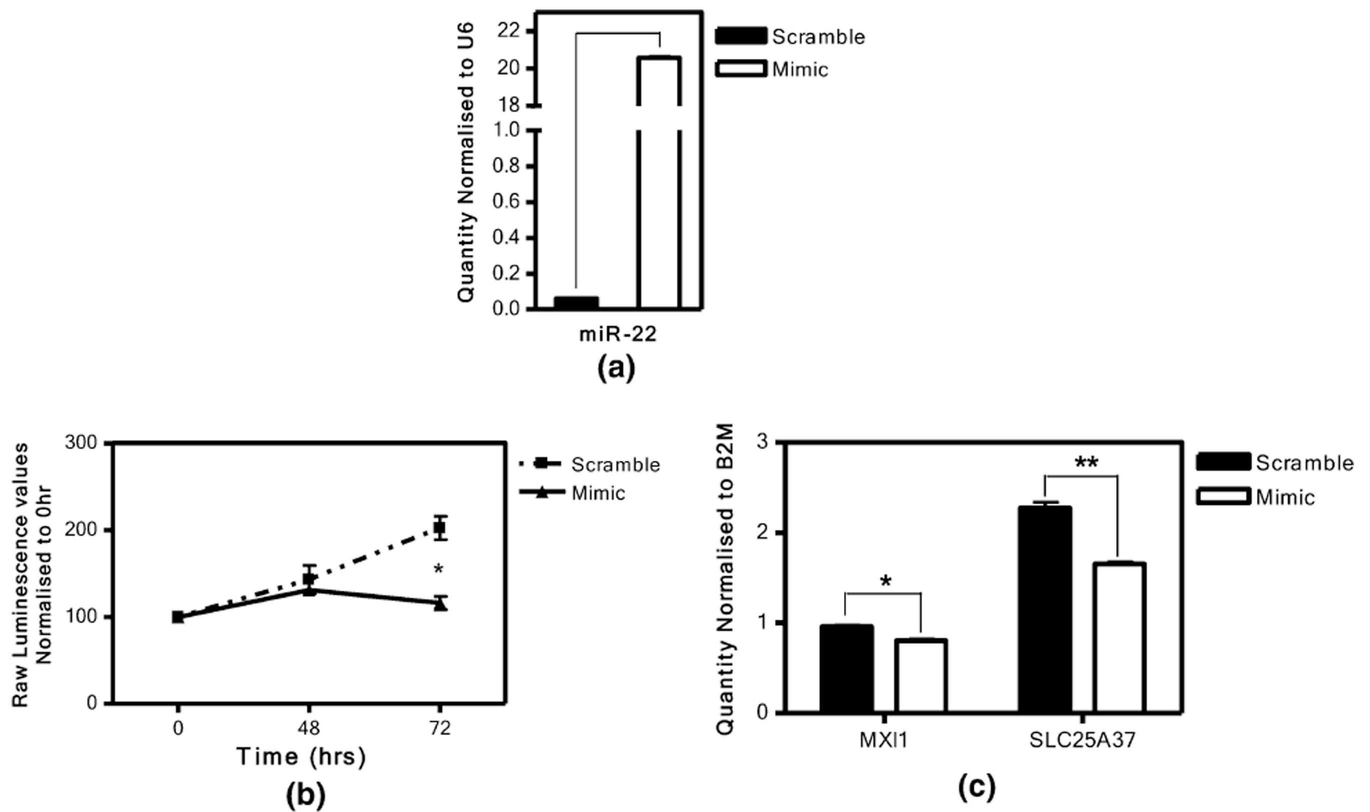

Fig. 4.

The effect of miR-22 over-expression in SKOV3 cell line. **a** Relative miR-22 expression level 24 h post-transfection. *RNU6* was used as housekeeping control. The transfection of miR-22 mimic (over-expression) resulted in increased expression of miR-22 at 24 h post-transfection in comparison to that of transfected scramble control (t test $p = 2.4 \times 10^{-05}$). **b** Significant inhibition of cell growth observed in SKOV3 at 72 h post-transfection of miR-22 mimic (t test $p = 0.0014$). Cellular growth was determined using CellTiter Glo assay. **c** Relative *MXI1* and *SLC25A37* expression levels 24 h post-addition of miR-22 mimic ($p = 0.003$ and $p = 0.001$, respectively). *B2M* was used as housekeeping control for PCR. “mimic” represents over-expression while “scramble” represents the control experiment

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Table 1

miRNA whose expression levels are significantly correlated with iGrowth

miRNA	iGrowth correlation p value			
	Pooled analysis		YRI	CEU
	Raw p value	*FDR*		
hsa-miR-210	3.51E−09	7.05E−07	1.41E−06	4.31E−04
hsa-miR-768-5p	1.39E−07	1.40E−05	6.51E−06	2.44E−03
hsa-miR-18a	2.55E−07	1.71E−05	1.53E−06	9.21E−03
hsa-miR-106a	8.59E−07	4.32E−05	1.75E−08	1.75E−01
hsa-miR-22	1.70E−06	6.13E−05	9.83E−08	6.73E−02
hsa-miR-18b	1.83E−06	6.13E−05	2.48E−06	3.72E−02
hsa-miR-17	2.81E−06	8.07E−05	5.58E−08	2.37E−01
hsa-miR-765	4.18E−06	1.05E−04	4.72E−05	3.22E−02
hsa-miR-20a	1.04E−05	2.30E−04	3.93E−07	1.53E−01
hsa-miR-20b	1.14E−05	2.30E−04	6.86E−06	1.42E−01
hsa-miR-1	1.32E−05	2.41E−04	1.91E−05	1.16E−01
hsa-miR-148b	3.81E−05	6.39E−04	4.66E−03	2.93E−03
hsa-miR-130b	5.71E−05	8.82E−04	1.17E−04	9.05E−02
hsa-miR-600	8.09E−05	1.16E−03	1.05E−02	1.83E−03
hsa-miR-768-3p	1.10E−04	1.41E−03	5.80E−04	3.62E−02
hsa-miR-518a-5p/hsa-miR-527	1.14E−04	1.41E−03	4.16E−04	4.66E−02
hsa-miR-17*	1.19E−04	1.41E−03	1.92E−07	9.62E−01
hsa-miR-198	1.38E−04	1.54E−03	1.77E−04	1.21E−01
hsa-miR-92a	2.29E−04	2.32E−03	3.29E−07	7.28E−01
hsa-miR-30b*	2.31E−04	2.32E−03	9.91E−05	2.13E−01
hsa-miR-33a	2.62E−04	2.50E−03	4.63E−06	7.53E−01
hsa-miR-103	3.20E−04	2.92E−03	5.85E−06	8.42E−01
hsa-miR-361-3p	3.49E−04	3.05E−03	2.95E−04	1.46E−01
hsa-miR-196a*	3.83E−04	3.20E−03	3.53E−02	4.78E−03
hsa-miR-19a	6.46E−04	5.19E−03	1.51E−06	3.43E−01

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miRNA	iGrowth correlation *p* value			
	Pooled analysis		YRI	CEU
	Raw *p* value	*FDR*		
hsa-miR-516a-5p	6.90E−04	5.26E−03	7.53E−05	3.21E−01
hsa-miR-365	7.06E−04	5.26E−03	2.64E−02	1.01E−02
hsa-miR-19b	1.07E−03	7.71E−03	1.35E−05	2.73E−01
hsa-miR-519e*	1.12E−03	7.80E−03	4.90E−04	2.69E−01
hsa-miR-921	1.22E−03	8.03E−03	5.80E−04	2.99E−01
hsa-miR-146a	1.24E−03	8.03E−03	4.53E−04	3.38E−01
hsa-miR-193b	1.33E−03	8.36E−03	2.18E−02	2.72E−02
hsa-miR-185*	1.55E−03	9.21E−03	3.98E−02	6.89E−03
hsa-miR-301a	1.59E−03	9.21E−03	2.21E−03	2.30E−01
hsa-miR-193b*	1.60E−03	9.21E−03	2.08E−03	1.70E−01
hsa-miR-518c*	1.79E−03	9.86E−03	1.56E−04	5.03E−01
hsa-miR-34a	1.81E−03	9.86E−03	8.21E−02	8.00E−03
hsa-miR-106b	2.54E−03	1.34E−02	7.70E−04	3.76E−01
hsa-miR-223	2.73E−03	1.41E−02	1.89E−02	6.38E−02
hsa-miR-146b-5p	2.94E−03	1.46E−02	1.98E−03	3.24E−01
hsa-miR-93	2.99E−03	1.46E−02	2.94E−04	6.92E−01
hsa-miR-483-5p	3.41E−03	1.63E−02	6.70E−05	4.87E−01
hsa-miR-132	4.99E−03	2.33E−02	1.24E−02	2.15E−01
hsa-miR-423-3p	5.38E−03	2.46E−02	5.03E−05	9.82E−01
hsa-miR-342-3p	6.00E−03	2.68E−02	3.93E−04	6.91E−01
hsa-miR-30c-2*	7.80E−03	3.41E−02	1.47E−03	6.84E−01
hsa-miR-675	8.37E−03	3.58E−02	4.12E−03	4.20E−01
hsa-miR-637	8.89E−03	3.65E−02	5.49E−01	5.75E−03
hsa-let-7d	8.90E−03	3.65E−02	1.20E−01	2.83E−02
hsa-miR-129*	9.51E−03	3.82E−02	6.01E−02	7.92E−02
hsa-miR-583	1.11E−02	4.36E−02	8.03E−04	9.82E−01
miRPlus_17952	1.18E−02	4.58E−02	7.13E−04	8.52E−01
hsa-miR-148a	1.30E−02	4.94E−02	4.31E−04	7.58E−01

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Only those miRNAs whose expression are correlated with iGrowth in pooled CEU and YRI samples are shown here (FDR < 0.05). miRNAs in bold have iGrowth correlation in both CEU and YRI samples with p value <0.05