RESEARCH ARTICLE

Implication of miR-612 and miR-1976 in the regulation of TP53 and CD40 and their relationship in the response to specific weight-loss diets

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Abstract

Background
Non-coding RNAs (i.e., miRNAs) play a role in the development of obesity and related comorbidities and the regulation of body weight.

Objective
To identify candidate miRNA biomarkers throughout omics approaches in order to predict the response to specific weight-loss dietary treatments.

Design
Genomic DNA and cDNA isolated from white blood cells of a subset from the RESMENA nutritional intervention study (Low-responders (LR) vs High-responders (HR)) was hybridized in Infinium Human Methylation450 BeadChip and in Illumina Human HT-12 v4 gene expression BeadChips arrays respectively. A bioinformatic prediction of putative target sites of selected miRNAs was performed by applying miRBase algorithms. HEK-293T cells were co-transfected with expression vectors containing the 3’-UTR of candidate genes to validate the binding of miRNAs to its target sites.

Results
134 miRNAs were differentially methylated between HR and LR in the methylation array, whereas 44 miRNAs were differentially expressed between both groups in the expression array. Specifically, miR-1237, miR-1976, miR-642, miR-636, miR-612 and miR-193B were simultaneously hypomethylated and overexpressed in HR. miR-612 and miR-1976 showed greatest differences in methylation and expression levels, respectively. The bioinformatic prediction revealed that TP53 was a putative target gene of miR-612 and CD40 of miR-
1976. Moreover, TP53 was downregulated in the expression array when comparing HR vs LR expression levels adjusted by sex, diet, age and baseline weight, and CD40 showed a statistical trend. Furthermore, gene expression levels of TP53 and CD40 in white blood cells, when measured by qPCR, were also downregulated in HR. Finally, miR-612 and miR-1976 potently repressed TP53 and CD40 respectively by targeting its 3'-UTR regions.

**Conclusion**

miR-612 and miR-1976 levels could be prospective biomarkers of response to specific weight-loss diets and might regulate the gene expression of TP53 and CD40.

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**Introduction**

The rates of obesity incidence have doubled in many countries since 80’s [1]. This growing prevalence and the related disease burdens highlight the need to understand not only the involved process but also to identify and evaluate biomarkers to address this problem. In the last decades, several types of biomarkers have been investigated in relation to their potential application in cardiovascular disease, infections, immunological and genetic disorders, and cancer diagnosis and management [2]. Concerning obesity, different biomarkers of response to different dietary approaches have been identified [3–5].

MicroRNAs (miRNAs) are non-coding RNAs (ncRNAs) of 18–25 nucleotides in length, that bind to a 3'-UTR target mRNA resulting usually in post-transcriptional regulation of gene expression [6] via transcript degradation (when the complementarity is nearly perfect) and/or inhibition of translation or deadenylation (when there are some mismatches in the binding).

On the other hand, miRNAs isolated from white blood cells [7] or directly from the circulation [8] are a good source of biomarkers, and their implication in obesity-related diseases has been well documented [9]. The identification of reliable biomarkers of response to anti-obesity treatments is of crucial importance in order to boost motivation, increase weight loss and maintenance success, and save time and money [10]. In this context, the use of biomarkers that predict the efficacy of weight loss treatments is considered a milestone in the design of precision nutrition strategies against obesity and related comorbidities [11].

In this study, we aimed to identify miRNAs from blood white cells that could be predictive of the outcome of a specific weight-loss intervention. For this purpose, in order to evaluate the interaction between dietary patterns targeting obesity and related transcriptomic biomarkers (miRNAs), we used a miRNAomic approach including methylation and expression microarrays.

**Materials and methods**

**Subjects and study protocol**

The current study was conducted in a subsample of the RESMENA (Metabolic Syndrome Reduction in Navarra) nutritional intervention trial. In this study, 96 adults with metabolic syndrome underwent two energy-restricted dietary patterns (AHA diet as reference diet, and RESMENA diet as intervention diet) during 8 weeks, both with an energy restriction of -30% of the studied requirements [12]. As no differences were found neither in anthropometric or biochemical variables between groups after the intervention, both dietary groups were merged for further analyses to increase the statistical power of the study, classifying subjects in “high-
responders” (HR) when weight loss was ≥8%, and “low responders” (LR) when weight loss was ≤8%, as previously published [4].

The study was performed following the CONSORT 2010 guidelines and properly approved by the Ethics Committee of the University of Navarra (065/2009) and registered at www.clinicaltrials.gov (NTC01087086). All participants provided written informed consent for participation.

**Microarray analyses**
Genomic DNA isolated from white blood cells of a subpopulation (31 LR vs 16 HR) of the RESMENA cohort was hybridized in an Infinium HumanMethylation450 BeadChip array (Illumina HM450K). Also, RNA from the same cells (14 LR vs 10 HR) was reverse transcribed and hybridized in an Illumina Human HT-12 v4 gene expression BeadChip array. Microarray data were analysed using Limma package in R [13]. Corrections for multiple comparisons were carried out in both microarrays (expression and methylation) by using the Benjamini-Hochberg procedure.

**Bioinformatic study**
A bioinformatic study of putative target sites of selected miRNAs was performed by applying miRBase algorithms (www.mirbase.org). For each miRNA, miRBase provides references in literature, genomic coordinates and links to databases of predicted and validated miRNA target sites such as DIANA-microT, microRNA.org, miRDB, RNA22, TargetMiner and TargetScan [14].

**MassArray Epityper validation**
In order to validate the results of the methylation microarray, miR-612 methylation levels of 47 subjects selected from the subpopulation sample were analyzed by MassArray EpiTyp0065r (Sequenom, San Diego, CA, USA) after designing specific primers encompassing 6 CpGs sites (F: GTTTTATGGTAGTGGAAGGGATTT; R: AATAAAACCCAAACAACAAACAAATC). This method has been previously applied to validate methylation microarray data [15].

**Luciferase reporter constructs**
To verify if selected miRNAs regulate the 3’-UTR of the two predicted target genes, expression vectors containing the 3’-UTR region of each gene provided by the bioinformatic prediction were designed. To amplify the 3’-UTR region of TP53 and CD40 genes, specific primers incorporating Nhel and XbaI restriction enzymes sites were designed. The PCR products were subsequently cloned downstream of the firefly luciferase (luc) gene in the pmiR-GLO Dual-Luciferase miRNA Target Expression Vector (Promega, Madison, WI, USA) (S1 Fig). Primer sequences are shown in Table 1.

| Primer Sequences | Amplicon Length |
|------------------|-----------------|
| CD40-F           | 5’-TTTAGCTAGCAGCTTATCATGATGCCAACACC-3’ | 766 bp |
| CD40-R           | 5’-TTATCTAGACACCCCTCTGAGCTTG-3’         |
| TP53-F           | 5’-TTAGCTAGCGCAACCCTCTGAGCTTG-3’        | 1010 bp |
| TP53-R           | 5’-TTATCTAGACACCCCTCTGAGCTTG-3’         |

F: Forward. R: Reverse. Underlined: Nhel and XbaI target sites

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Cell culture
Human HEK-293T cells were purchased from the ATCC and maintained in Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 100 U/ml penicillin-streptomycin at 37°C in a 5% carbon dioxide humidified atmosphere.

Dual-luciferase reporter assays
To assess miRNA-target interactions, HEK-293T cells were seeded in 96-well plates at a density of 20000 cells per well. After 8 h, cells were transiently co-transfected with either 0.25 μg of empty pmiR-GLO, pmiR-GLO-TP53-3’-UTR, or pmiR-GLO-CD40-3’-UTR, and 7.5 pmol of miR-612 and miR-1976 mimics using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to manufacturer’s protocol. Firefly and Renilla luciferase activities were evaluated 24 h after co-transfection using a Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA). Firefly luciferase activity was normalized using Renilla luciferase activity. Determinations were carried out in three independent experiments, each assayed in triplicate.

RNA isolation and quantitative real-time PCR
RNA from white blood cells was extracted using TRIzol reagent (Life Technologies, Carlsbad, CA, USA) according to manufacturer’s protocol. cDNA was synthesized from 0.5 μg of total RNA using random primers and MultiScribe™ MuLV reverse transcriptase (Applied Biosystems, Foster City, CA, USA). For mature miR-1976, 20 ng of RNA were reverse transcribed by using a Taqman MicroRNA RT kit (Applied Biosystems) and miRNA-specific primer sets supplied by the manufacturer. Quantitative real time PCR (qPCR) was performed with the ABI prism 7900HT Sequence Detection System (Applied Biosystems) using Taqman probes. Both mRNAs and miRNAs relative expression was calculated with the $2^{-ΔΔCT}$ method and normalized using GAPDH and U48 mature miRNA, respectively.

Statistical analysis
Differences between groups were calculated using the Student’s t-test or an ANCOVA test when indicated. Data are presented as mean ± SEM. p-values less than 0.05 were defined as statistically significant. Volcano plots were created by plotting the negative log$_{10}$ of the p-value (y axis) and the mean differences between groups for each variable (x axis). An effect size of ±1.5% in the methylation differences and an effect size of ±1% in the expression differences were considered of interest. Statistical analyses and graphics were performed using SPSS 15.0 software (SPSS Inc., Chicago, IL, USA) and GraphPad Prism version 6.0C (La Jolla, CA, USA).

Results
miR-612 and miR-1976 are hypomethylated and overexpressed in HR
When analyzing array data, 134 miRNAs differentially methylated (87 hypomethylated and 47 hypermethylated), and 44 miRNAs differentially expressed (10 downregulated and 34 upregulated) were identified when comparing HR and LR (S1 and S2 Tables). From them, miR-1237, miR-1976, miR-612, miR-636, miR-612 and miR-193B were simultaneously hypomethylated and overexpressed in HR (Fig 1A and 1B). miR-612 showed significant difference in expression levels (1.43% effect size; p = 0.019) and the greatest difference in methylation levels (10% effect size; p = 0.003). Likewise, miR-1976 showed also significant difference in methylation levels (3.24% effect size; p = 0.041) and greatest differences in expression levels (12% effect size; p = 0.043). Although nominal statistically differences in miR-612 and miR-1976 between HR and LR were found, they disappeared after correction for multiple comparisons.
Nevertheless, as these miRNAs showed notable differences in both methylation and expression microarrays, they were selected for further evaluation.

**TP53 is a putative target gene of miR-612**

To select putative miRNAs implicated in the response to the diet, a bioinformatic study using miRBase algorithms assigning p-values to putative target binding sites of those miRNAs simultaneously hypomethylated and overexpressed in arrays was carried out. We then focused on obesity-related genes and filtered the target sites of each miRNA. We noted that TP53 was predicted to be regulated by miR-612. EpiTyper analysis of the DNA methylation levels of miR-612 in white blood cells showed a positive correlation ($p < 0.001$) with respect to the DNA methylation levels measured by microarray (Fig 2A). Moreover, TP53 was downregulated in the expression array ($p = 0.024$) when comparing HR vs LR expression levels adjusted by sex, diet, age and baseline weight (Fig 2B). Furthermore, TP53 levels were also significantly lower in HR than in LR when measured by qPCR ($p = 0.04$), supporting the idea that miR-612 might affect TP53 gene expression (Fig 2C). Finally, cells co-transfected with the pmiR-GLO-TP53-3'-UTR vector and miR-612 showed significantly lower firefly/Renilla activity ($p < 0.001$) than controls transfected only with the pmiR-GLO-TP53-3'-UTR vector (Fig 2D), confirming that TP53 is a target gene of miR-612.

**CD40 is a putative target gene of miR-1976**

Similarly to miR-612, we carried out the same approach to identify putative obesity-related target genes of miR-1976. According to miRBase, CD40 could be regulated by miR-1976. First, we found that miR-1976 expression profile in white blood cells was statistically different between HR and LR ($p = 0.019$) and also showed a positive correlation ($p = 0.012$) with miR-1976 expression levels measured by microarray (Fig 3A–3B). CD40 expression level showed a trend toward significance ($p = 0.069$) when comparing HR vs LR adjusted by sex, diet, age and baseline weight (Fig 3C). Interestingly, gene expression levels of CD40 were also significant negatively correlated ($p = 0.023; R = -0.505$) with miR-1976 expression profile (Fig 3D).
Furthermore, CD40 expression in white blood cells, when measured by qPCR, were significantly lower in HR than in LR (p = 0.02) (Fig 3E), suggesting an interaction between miR-1976 and CD40. Similarly, cells co-transfected with the pmiR-GLO-CD40-3’-UTR construct and miR-1976 showed also a significantly reduction in firefly/Renilla activity (p = 0.014) than controls transfected only with the expression vector (Fig 3F), confirming that CD40 is a target gene of miR-1976.

**Discussion**

In this study, a miRNAomic approach was performed in order to find transcriptomic biomarkers (especially miRNAs) associated to the response to specific weight loss diets. It is well established that miRNAs can regulate the expression of genes by binding to its target sites, usually resulting in degradation or translation inhibition [6]. miRNAs have been implicated in many development and diseases processes [16], including obesity and associated comorbidities [17]. For example, in type 2 diabetes, a well-known obesity-related disease, several miRNAs are down or upregulated [18]. miR-103 and miR-107 have been reported to contribute to adipose growth by accelerating adipocyte differentiation, and both are upregulated in obese individuals [19]. Moreover, several miRNAs have been defined as important modulators in human
obesity-related inflammation [20] or white adipose tissue inflammation [21], adipogenesis and adipose tissue signaling [22]. Additionally, changes in miRNA levels in plasma, serum, urine and other fluids have been associated with different diseases such as prostate cancer [23], bladder cancer [24] or cell carcinoma [25]. Thus, the identification of circulating miRNAs could serve as useful clinical biomarkers of diagnosis and prognosis of several diseases [26].

The present study has demonstrated that miR-612 and miR-1976 bind to TP53 and CD40 respectively and regulate their expression. TP53 gene encodes p53 protein, a tumor suppressor whose deficiency enhances the initiation and/or progression of cancer [27]. Noticeably, Minamino et al. found that the expression of proinflammatory cytokines in mice decreased and insulin resistance improved after inhibition of p53 in adipose tissue, suggesting an important role of p53 in the regulation of obesity-related inflammation and insulin resistance [28]. Furthermore, they also evidenced that adipose tissue from subjects with diabetes showed higher levels of p53 protein compared with tissue from nondiabetic subjects, and that the expression of inflammatory cytokines was also significantly increased in adipose tissue.

Moreover, ob/ob mice show higher levels of p53 than wild type mice, and the disruption of p53 in ob/ob mice restores the expression of lipogenic enzymes [29]. Conversely, Molchadsky et al. showed that p53 may exert either a positive or negative effect according to the adipogenic differentiation program [30]. In our study, those subjects who responded better to the diet had lower expression of TP53 than LR. It can be speculated that LR had higher inflammatory state than HR, and that this inflammation could trigger an activation of TP53.
In white blood cells, TP53 is downregulated in obese subjects with type 2 diabetes after bariatric surgery, suggesting that TP53 is upregulated in white blood cells of obese subjects [31]. Taking all these data together, our findings of decrease whole blood TP53 mRNA in HR are consistent with these studies.

Several TP53-directed miRNAs have been experimentally and in silico identified. Some of these miRNAs that target TP53 are miR-125b, miR-504, miR-1285, miR-92, miR-141, miR-380-5p, miR-15a, miR-16, miR-25, miR-30d, miR-200a, miR-453, miR-98, miR-19b, miR-518c and miR-638 [32]. Interestingly, miR-1285 has the same seed sequence as miR-612. In a previous study, Tian et al. tried to validate the binding of miR-612 to TP53 and found that the luciferase activity of the p-LUC-p53-3’-UTR reporter did not change when transiently transfected with miR-612 [33]. However, in the present study, our data show that miR-612 binds to the 3’-UTR of TP53 and that there exists a negative relationship between miR-612 levels and TP53 expression (in blood and in the microarray), indicating that miR-612 could regulate TP53 expression.

On the other hand, CD40 is a surface glycoprotein expressed in hematopoietic and nonhematopoietic cells, and has an important role in the ability to stimulate adaptive immunity [34]. Concerning obesity, CD40 is highly expressed in leukocytes, adipocytes and the stromal cells of adipose tissue [35], and is involved in the regulation of adipose tissue metabolism [36]. Furthermore, soluble CD40L levels have been positively correlated with obesity and metabolic syndrome [37,38], and studies in rodents have shown that vascular inflammation and atherosclerosis could be prevented by CD40 deficiency [39,40]. There exists evidence that CD40 could be regulated by miRNAs [41,42], even though to date there are no articles showing a miRNA-regulation of CD40 in obese subjects.

To our knowledge, this is the first study to explore the relationships between miR-612 and miR-1976 and TP53 and CD40, respectively, and was able to connect the expression of these miRNAs and genes with the response to a dietary intervention in obese subjects. However, further studies are needed to better understand the complex regulation of these miRNAs on their target genes. Transcriptomic biomarkers of the response to specific dietary strategies are a first step towards the personalization of weight-loss treatment, being miRNAs particularly relevant for this purpose.

Supporting information

S1 Fig. miR-612 and miR-1976 regulate the 3’-UTR region of TP53 and CD40, respectively. A) Location of putative target sites for miR-612 and miR-1976 in the 3’-UTR of TP53 and CD40 predicted by TargetScan. B) miR-GLO Dual-Luciferase miRNA Target Expression Vector used to create the 3’-UTR expression vectors cloning the PCR product into the MCS. MCS: Multiple Cloning Site.

(PDF)

S1 Table. Significantly differentiated methylated miRNAs between HR and LR. In bold style, those miRNAs that were above the selected threshold of ±1.5%.

(DOCX)

S2 Table. Significantly differentiated expressed miRNAs between HR and LR. In bold style, those miRNAs that were above the selected threshold of ±1%.

(DOCX)

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Author Contributions

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References

1. Collaborators TGO (2017) Health Effects of Overweight and Obesity in 195 Countries over 25 Years. N Engl J Med.
2. Mayeux R (2004) Biomarkers: Potential Uses and Limitations. NeuroRx 1: 182–188. https://doi.org/10.1602/neurorx.1.2.182 PMID: 15717018
3. Campion J, Milagro FI, Goyenechea E, Martinez JA (2009) TNF-alpha promoter methylation as a predictive biomarker for weight-loss response. Obesity (Silver Spring) 17: 1293–1297.
4. Garcia-Lacarte M, Milagro FI, Zulet MA, Martinez JA, Mansego ML (2016) LINE-1 methylation levels, a biomarker of weight loss in obese subjects, are influenced by dietary antioxidant capacity. Redox Rep 21: 67–74. https://doi.org/10.1179/1351000215Y.0000000029 PMID: 26197243
5. Cordero P, Campion J, Milagro FI, Goyenechea E, Steemburgo T, et al. (2011) Leptin and TNF-alpha promoter methylation levels measured by MSP could predict the response to a low-calorie diet. J Physiol Biochem 67: 483–470. https://doi.org/10.1007/s13105-011-0084-4 PMID: 21465273
6. Filipowicz W, Bhattacharya SN, Sonenberg N (2008) Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? Nat Rev Genet 9: 102–114. https://doi.org/10.1038/nrg2290 PMID: 18197166
7. Milagro FI, Miranda J, Portillo MP, Fernandez-Quintela A, Campion J, et al. (2013) High-throughput sequencing of microRNAs in peripheral blood mononuclear cells: identification of potential weight loss biomarkers. PLoS One 8: e54319. https://doi.org/10.1371/journal.pone.0054319 PMID: 23335998
8. Creemers EE, Tijsen AJ, Pinto YM (2012) Circulating microRNAs: novel biomarkers and extracellular communicators in cardiovascular disease? Circ Res 110: 483–495. https://doi.org/10.1161/CIRCRESAHA.111.247452 PMID: 22302755
9. Marques-Rocha JL, Sambias M, Milagro FI, Bressan J, Martinez JA, et al. (2015) Noncoding RNAs, cytokines, and inflammation-related diseases. FASEB J 29: 3595–3611. https://doi.org/10.1096/fj.14-260323 PMID: 26065857
10. Ramos-Lopez O, Milagro FI, Allayee H, Chmurzynska A, Choi MS, et al. (2017) Guide for Current Nutrigenetic, Nutrigenomic, and Nutriepigenetic Approaches for Precision Nutrition Involving the Prevention and Management of Chronic Diseases Associated with Obesity. J Nutrigenet Nutrigenomics 10: 43–62. https://doi.org/10.1159/000477729 PMID: 28689206
11. Goni L, Cuervo M, Milagro FI, Martinez JA (2016) Future Perspectives of Personalized Weight Loss Interventions Based on Nutrigenetic, Epigenetic, and Metagenomic Data. J Nutr.
12. Zulet MA, Bondía-Pons I, Abete I, de la Iglesia R, Lopez-Legarrea P, et al. (2011) The reduction of the metabolytic syndrome in Navarra-Spain (RESMENA-S) study: a multidisciplinary strategy based on chrononutrition and nutritional education, together with dietary and psychological control. Nutr Hosp 26: 16–26.
13. Ritchie ME, Shipson B, Wu D, Hu Y, Law CW, et al. (2015) limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res 43: e47–e47. https://doi.org/10.1093/nar/gkv079 PMID: 26505792
14. Kozomara A, Griffiths-Jones S (2014) miRBase: annotating high confidence microRNAs using deep sequencing data. Nucleic Acids Res 42: D68–D73. https://doi.org/10.1093/nar/gkt181 PMID: 24275495
15. Mansego ML, Garcia-Lacarte M, Milagro FI, Martí A, Martinez JA, et al. (2017) DNA methylation of miRNA coding sequences putatively associated with childhood obesity. Pediatric Obesity 12: 19–27. https://doi.org/10.1111/jpo.12101 PMID: 26780939
16. Sayed D, Abdelatif M (2011) MicroRNAs in development and disease. Physiol Rev 91: 827–887. https://doi.org/10.1152/physrev.00006.2010 PMID: 21742789
17. Peng Y, Yu S, Li H, Xiang H, Peng J, et al. (2014) MicroRNAs: emerging roles in adipogenesis and obesity. Cell Signal 26: 1888–1896. https://doi.org/10.1016/j.cellsig.2014.05.006 PMID: 24844591
18. McClelland AD, Kantharidis P (2014) microRNA in the development of diabetic complications. Clin Sci (Lond) 126: 95–110.
19. Xie H, Lim B, Lodish HF (2009) MicroRNAs induced during adipogenesis that accelerate fat cell development are downregulated in obesity. Diabetes 58: 1050–1057. https://doi.org/10.2337/db08-1299 PMID: 19188425
20. Zhang XM, Guo L, Chi MH, Sun HM, Chen XW (2015) Identification of active miRNA and transcription factor regulatory pathways in human obesity-related inflammation. BMC Bioinformatics 16: 76. https://doi.org/10.1186/s12859-015-0152-5 PMID: 25887648
21. Ge Q, Brichard S, Yi X, Li Q (2014) microRNA as a new mechanism regulating adipose tissue inflammation in obesity and as a novel therapeutic strategy in the metabolic syndrome. J Immunol Res 2014: 987285. https://doi.org/10.1155/2014/987285 PMID: 24741638
22. Arner P, Kulyte A (2015) MicroRNA regulatory networks in human adipose tissue and obesity. Nat Rev Endocrinol 11: 276–288. https://doi.org/10.1038/nrendo.2015.25 PMID: 25732520
23. Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, et al. (2008) Circulating microRNA as stable blood-based markers for cancer detection. Proc Natl Acad Sci U S A 105: 10513–10518. https://doi.org/10.1073/pnas.0804549105 PMID: 18663219
24. Hanke M, Hoefig K, Merz H, Feller AC, Kausch I, et al. (2010) A robust methodology to study urine microRNA as tumor marker: microRNA-126 and microRNA-182 are related to urinary bladder cancer. Urol Oncol 28: 655–661. https://doi.org/10.1016/j.urolonc.2009.01.027 PMID: 19375957
25. Park NJ, Zhou H, Elashoff D, Henson BS, Kastratovic DA, et al. (2009) Salivary microRNA: discovery, characterization, and clinical utility for oral cancer detection. Clin Cancer Res 15: 5473–5477. https://doi.org/10.1158/1078-0432.CCR-09-0736 PMID: 20187728
26. Etheridge A, Lee I, Hood L, Galas D, Wang K (2011) Extracellular microRNA: a new source of biomarkers. Mutation research 717: 85–90. https://doi.org/10.1016/j.mrfmmm.2011.03.004 PMID: 21402084
27. Bieging KT, Mello SS, Attardi LD (2014) Unravelling mechanisms of p53-mediated tumour suppression. Nat Rev Cancer 14: 359–370. https://doi.org/10.1038/nrcc3711 PMID: 24739573
28. Minamino T, Orimo M, Shimizu I, Kunieda T, Yokoyama M, et al. (2009) A crucial role for adipose tissue factor regulatory pathways in human obesity-related inflammation. BMC Bioinformatics 16: 76. https://doi.org/10.1186/s12859-015-0152-5 PMID: 25887648
29. Yahagi N, Shimano H, Matsuzaka T, Najima Y, Sekiya M, et al. (2003) p53 Activation in adipocytes of obese mice. J Biol Chem 278: 25395–25400. https://doi.org/10.1074/jbc.M302364200 PMID: 12734185
30. Molchadsky A, Ezra O, Amendola PG, Krantz D, Kogan-Sakin I, et al. (2013) p53 is required for brown adipogenic differentiation and has a protective role against diet-induced obesity. Cell Death Differ 20: 774–783. https://doi.org/10.1038/cdd.2013.9 PMID: 23412343
31. Berisha SZ, Serre D, Schauer P, Kashyap SR, Smith JD (2011) Changes in whole blood gene expression following bariatric surgery: a pilot study. PLoS One 6: e16729. https://doi.org/10.1371/journal.pone.0016729 PMID: 21423737
32. Vijayakumaran R, Tan KH, Miranda PJ, Haupt S, Haupt Y (2015) Regulation of Mutant p53 Protein Expression. Front Oncol 5: 284. https://doi.org/10.3389/fonc.2015.00284 PMID: 26734569
33. Tian S, Huang S, Wu S, Guo W, Li J, et al. (2010) MicroRNA-1285 inhibits the expression of p53 by directly targeting its 3’ untranslated region. Biochem Biophys Res Commun 390: 435–439. https://doi.org/10.1016/j.bbrc.2010.04.112 PMID: 20417621
34. Fuji S-I, Liu K, Smith C, Bonito AJ, Steinman RM (2004) The Linkage of Innate to Adaptive Immunity via Maturing Dendritic Cells In Vivo Requires CD40 Ligation in Addition to Antigen Presentation and CD80/86 Costimulation. J Exp Med 199: 1607–1618. https://doi.org/10.1084/jem.20040317 PMID: 15197224
35. Poggi M, Jager J, Paulmyer-Lacroix O, Peiretti F, Gremeaux T, et al. (2009) The inflammatory receptor CD40 is expressed on human adipocytes: contribution to crosstalk between lymphocytes and adipocytes. Diabetologia 52: 1152–1163. https://doi.org/10.1007/s00125-009-1267-1 PMID: 19183933
36. Chatzigeorgiou A, Phieler J, Gebler J, Bornstein SR, Chavakis T (2013) CD40L stimulates the crosstalk between adipocytes and inflammatory cells. Horm Metab Res 45: 741–747. https://doi.org/10.1055/s-0033-1348221 PMID: 23918687
37. Unek I1, Bayraktar F, Solmaz D, Ellidokuz H, Sisman AR, et al. (2010) The levels of soluble CD40 ligand and C-reactive protein in normal weight, overweight and obese people. Clin Med Res 8: 89–95. https://doi.org/10.3121/cmr.2010.889 PMID: 20660932
38. Unek IT, Bayraktar F, Solmaz D, Ellidokuz H, Yuksel F, et al. Enhanced levels of soluble CD40 ligand and C-reactive protein in a total of 312 patients with metabolic syndrome. Metabolism—Clinical and Experimental 59: 305–313. https://doi.org/10.1016/j.metabol.2009.04.034 PMID: 20006362
39. Mach F, Schonbeck U, Sukhova GK, Atkinson E, Libby P (1998) Reduction of atherosclerosis in mice by inhibition of CD40 signalling. Nature 394: 200–203. https://doi.org/10.1038/28204 PMID: 9671306
40. Lutgens E, Lievens D, Beckers L, Wijnands E, Soehnlein O, et al. (2010) Deficient CD40-TRAF6 signalling in leukocytes prevents atherosclerosis by skewing the immune response toward an antiinflammatory profile. J Exp Med 207: 391–404. https://doi.org/10.1084/jem.20091293 PMID: 20100871
41. Guo X, Li D, Chen M, Chen L, Zhang B, et al. (2016) miRNA-145 inhibits VSMC proliferation by targeting CD40. Sci Rep 6: 35302. https://doi.org/10.1038/srep35302 PMID: 27731400
42. Luo S, Liu Y, Liang G, Zhao M, Wu H, et al. (2015) The role of microRNA-1246 in the regulation of B cell activation and the pathogenesis of systemic lupus erythematosus. Clin Epigenetics 7: 24. https://doi.org/10.1186/s13148-015-0063-7 PMID: 25789080