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Original article

To investigate the expression of miRNA in nicotine induced GISTs tumor rats and the mitigation of cardiovascular complications

Yu Li [a], Narendra Maddu [b], Umeshareen Shaik [c], Fareeda Begum Shaik [b], Guangping Zhang [d,*]

[a] Department of Electrophysiology, 3201 Hospital, Hanzhong 723000, China
[b] Department of Biochemistry, Sri Krishnadevaraya University, Ananthapuram -515003, Andhra Pradesh, India
[c] Sri Adi Siva Sadguru Alli Saheb Sivaaryula Ayurvedic Medical College, Guntakal, A.P., India
[d] Department of Cardiology, Shaanxi Province Hospital of Traditional Chinese Medicine, 710003, Xi'an, China

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ABSTRACT

The common for the most part of tumor (GISTs) Gastrointestinal stromal tumor is known mesenchymal neoplasms of the tumor in gastrointestinal tract. Detection of serum miRNA 1, 15b, 21 adjustment mainly cardio marker enzymes and their mechanism of action and heart tissue, observed changes in LDH, CK-MB, AST, ALT, lipid peroxidation (LPO) and Troponin T estimations/biochemical changes observed in serum of experimental subjects (nicotine treated rats, 0.6 mg/kg, i.p.). In the present study, gastrointestinal stromal tumors of rats and the mitigation of cardiovascular complications was experimentally induced in rats by nicotine i.p. treatment of adult rats and maintained control rats. Confirm significantly decreased expression of miRNA-1 (nicotine induced gastrointestinal tumor rats), the levels of miRNA-15b expression is decrease in group II but miRNA-21 expression values are increased in experimental rats (group II) with control rats (group I). Body weight decreased significantly in group II nicotine induced rats and heart weight increased in group II but systolic blood pressure is decreased in experimental rats (group II) with control rats. Cardio marker enzyme levels were measured and increases LDH levels, slight raises CK-MB concentration range in group II. AST, ALT marker enzyme levels are higher in experimental rats with controls, troponin T values are clearly declare that some cardio complications in group II. Observed Ldh, Ck-Mb, Ast and Alt levels are decreased followed by antioxidant enzymes SOD, catalase but lipidperoxidation and protein carbonyls values are increased in nicotine induced rats and match up to normal rats. GST levels are increases significantly and Gpx, GRd and GSH levels are decreased in group II nicotine induced rats and corporately control group I. This study to investigates, for the first time, the effect of nicotine i.p., in adult rats (nicotine treated) and control rats. Reports of serum as well as heart tissue adjustment in molecular levels.

1. Introduction

For the most part of regular neoplasms of mesenchymals are GISTs (Gastrointestinal stromal tumors)/gastroin. Usually GISTs are misidentifying as leiomyosarcomas or leiomyomas awaiting the report of (Miettinen and Virolainen, 1995) the revealed with the intention of the CD34 tumor expression and differentiate GISTs as of leiomyosarcomas, leiomyomas. At present, analysis of GISTs include develop into additional clear-cut all the way through staining of histochemical and immunological changes by the detection of exterior indicator CD117 with (95% of positive rate) & 95% of DOG1. Mostly GISTs are usually establish small intestine (25%), stomach (60%), even as roughly 5% are originate in the rectum or colon, and simply 2% in the other organs or esophagus (Corless et al., 2004). The scientific indication of GISTs includes nausea, pain of abdominal, dysphagia, and gastrointestinal flow of blood in chronic conditions. Oncogene is a kind of GISTs are metamorphosis ambitious growth, among ordinary alteration occur (80%-90%) in KIT gene, tyrosine kinase receptor, derived platelet expansion receptor factor alpha (5%) gene in PDGFRA (Hirota et al., 1998; Heinrich et al., 2003). New investigate has exposed links to BRAF and SDH transmutation (Agaimy et al., 2009; Hostein et al., 2010).

* Corresponding author.
E-mail address: m13636768006@sina.com (G. Zhang).

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A position of endogenous miRNAs, little RNAs non-coding range a piece of 19–24 nt, meaning from side to side translational hang-up or the dreadful conditions of intention mRNAs (Bartel, 2004). Well-known with the intention of miRNAs be input in adaptable pathways and genes, a range of natural networks (Bartel, 2004).

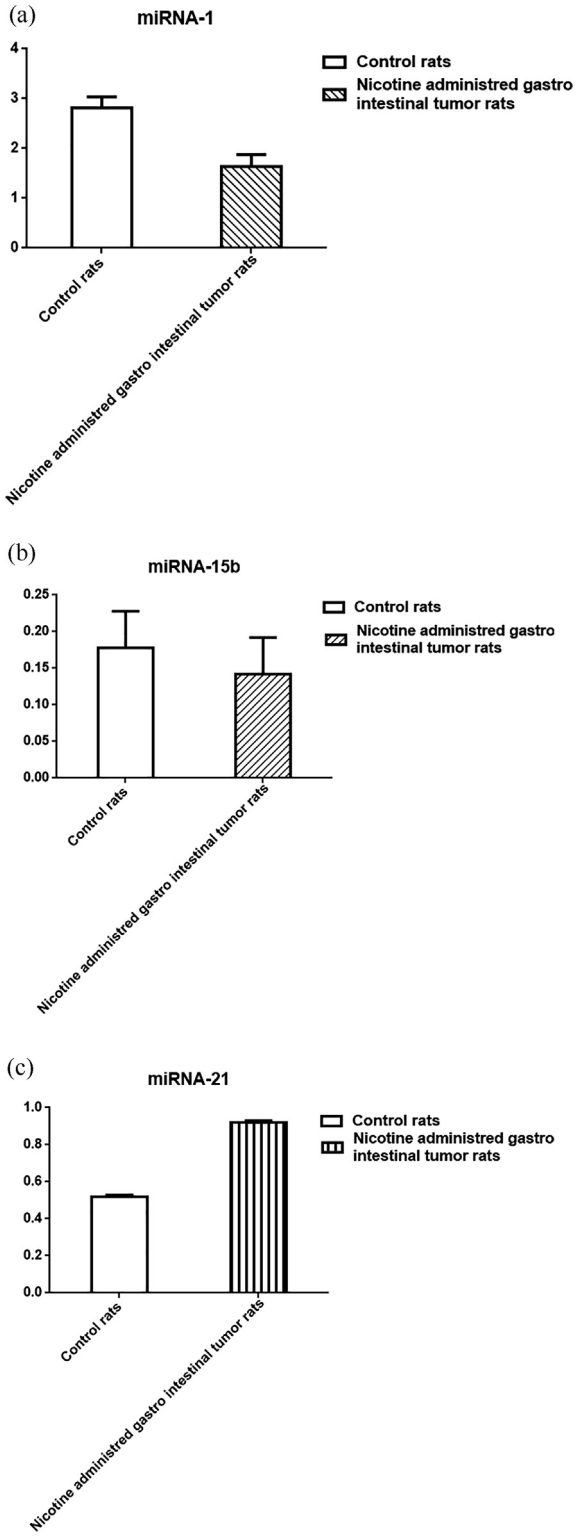

Fig. 1. Expression of miRNA-1, 15b, 21 levels in nicotine induced gastrointestinal tumors rats and control rats. Values are expressed as Mean ± SD, significantly different is set ($P \leq 0.05$) to Duncan's Multiple Range (DMR) test, n = 12. miRNA expression (Normalized by U6snRNA).

MiRNAs are being circulating and intensively scrutinize and their participation, a mixture of development and pathogenic, might serve up as possible syndrome biomarkers (Mitchell et al., 2008; Chen et al., 2008). The deliberation of miRNA circulating able to be precious issue, counting time and sex, and environmental factors, as well as livelihood situation, (Meder et al., 2014). Though, the appearance outline and method of miRNAs below hypoxia at far above the ground elevation stay to be completely revealed. Arrival of wide genome knowledge, as well as gene appearance microarrays, have complete it probable to get a widespread observation of miRNA modification related through elevated hypoxia, bioinformatics use and examination of the trail of the changed miRNAs. Hundreds to dozens of target genes are present in each miRNA control are separate. Therefore, miRNAs are expected more than third of protein-coding human genes expression and regulate in the recent report (Lewis et al., 2005).

At present 2500 more miRNAs are account inside individual (http://www.miRbase.org/). MiRNA participation is a critical position inside a variety of cell and molecular pathways. MiRNA play a significant role in gene regulation in post transcriptional in irradiated cells reported many studies (Templin et al., 2011; Lee et al., 2014). Furthermore, micro RNAs shows abnormal look outline in the common heart disease and a few number of specify that expression of miRNA well-organized marker diagnostic of heart complications/diseases are reported (van Rooij et al., 2006; Li et al., 2012). Present study were intended to examine the nicotine induced rats basis to serum miRNA 1, 15b, 21 adjustments particularly cardio marker enzymes and their mechanism of action in serum, AST, ALT, CK-MB, LDH, lipid peroxidation and Troponin T. We have also assessed heart tissue marker enzymes and modifications/changes in the levels of antioxidants SOD, Catalase, Lipidperoxidation, Protein carbonyls, GPx, GRd, GSH, and GST.

2. Methodology and experimental design

2.1. Animals procuring and design

Male albino *Wistar* strain rats twenty four old 60 days range body weight from l20-140 gm separate cage and quarters at room temp (25 + 1 °C) in (AC) Air Condition for the duration of 7 a.m to 7 p.m. The rats separated two sets each set 12 rats. **Group I** control rats the same quantity of purify water as an alternative of Nicotine, we develop the design a gastrointestinal stromal tumors **Group II** e. g. Nicotine induced gastrointestinal stromal tumors model rats alone (0.6 mg/kg, i.p.) body weight/day. The rats were validating every day mass and ingestion of foodstuff and water was following scheduled alternate time. At the end of the trial period, in each group of rats were fasting during the night plus in that case by utilize of cervical disturbance rats were sacrificed. Blood and organs were collected in addition to instantly used for extra examination for progression. This research was approved by Hanzhong 3201 Hospital, China, animal ethical committee, Approved No. HZ3201-13433.

2.2. Blood collection and sample study

2.2.1. Extraction of RNA by reverse transcription

Serum samples 200 µl was spiked with 25 fmol synthetic cel-miR-39, 2 µl was added (Tiangen, Beijing, China) while outside location. Whole RNA supplement isolated tiny RNAs was concurrently using microRNA Isolation Kit as of the serum with miRcute (Tiangen, Beijing, China) according to the tailored manufacturer's procedure (Chen et al., 2013). Purities and concentrations of miRNA was find out the, we make use of a NanoDrop, sample

absorbance was measured spectrophotometer (NanoDrop, Wilmington, DE, USA) at 260–280 nm.

The take out microRNA extraction was polyadenylated by poly (A) polymerase 20 μl. Add 6 μl of the poly (A) solution was reverse transcribed to cDNA, and 20 μl one more miRute miRNA and Kit with synthesis of first strand cDNA (Tiangen, Beijing, China) followed by reverse transcription was run in triplicate instructions of manufacturer's.

2.2.2. miRNA quantification by RT-PCR

PCR response be carry out on behalf of intensification with Detection Kit of miRcute qPCR microRNA (Tiangen, Beijing, China) Detection System 7500 Sequence of ABI PRISM (Applied Biosystems, Foster City, CA, USA). Every qPCR response explanation limited attenuate cDNA, and premix (with SYBR and ROX) 2 × miRcute microRNA, the manufacturer are give forward primers of microRNA-specific, a general reverse primer to a sum quantity of 20 μl. The qPCR response 94 °C pre-denaturation for 2 min were observed this parameters, 45 cycles of 20 s, 94 °C, 34 s 60 °C annealing, 30 s extension of 72 °C. A melt bend examination is consummate to make sure the specificity of the objective end of the PCR.

The relation appearance of microRNAs is designed by the equation $\log_{10}(2^{-\Delta CT})$. The ΔCT was equivalent to CT standards of the microRNAs of attention defect the CT standards of the cel-miR-39 (Chen et al., 2013).

2.2.3. Estimation of biochemical parameters

Creatinine kinase–MB were estimated by the method Rosalki (1967), amount of lactate dehydrogenase (LDH) by King (1965), AST (aspartate transaminase) and ALT (alanine transaminase) activities were estimated by the method Reitman and Frankel (1957) in serum and heart. By using immunoassay serum Troponin T was determined by Katus et al. (1991) it is extremely precise enzyme. Behavior of antioxidant (SOD) superoxide dismutase were determined by Kakkar et al. (1984), catalase estimation by technique of Maehly and Chance (1954), GPx (glutathione peroxidase) is measured Agergaard & Jensen (1982), GRd (glutathione reductase) by David & Richard (1983) GST (glutathione–S–transferase) and GSH (Reduced glutathione) were determined by the method Habig et al. (1974), Ellman (1959), estimation of protein carbonyls by Levine et al. (1994) in heart tissue were estimated.

2.2.4. Lipid peroxidation in serum

LPO is calculated through (MDA) malondialdehyde configuration with method (Sushama Kumari et al., 1990). 1 ml of serum was use in a test tube to which (0.375% w/v TBA, 15% w/v TCA & 0.25 N HCl) 2 ml of reagent was additional and reserved in hot stream wash for 15 min after surrounded by permitted to chill and then centrifuged 10 min at 1000*g*. Collected supernatant was move keen on other test tube; sample was examining at 535 nm by a spectrophotometer adjacent to the blank reagent presumptuous the 1.56 × 105 M extinction coefficient.

3. Results

Confirm significant decreased expression of miRNA-1 in group II experimental rats (nicotine induced gastrointestinal tumor rats), the levels of miRNA-15b expression is decrease in group II but miRNA-21 expression values are increased group II experimental rats with group I controls. MiRNA-1 expression was calculated in rat serum/tissues after treatment of nicotine for chronic period in Fig. 1a-1c. In Table 1 shows the data rat body weight decreased significantly in group II nicotine induced rats and heart weight increased in group II but systolic blood pressure is decreased in experimental rats (group II) compared to control rats. Cardio marker enzyme levels were measured and increases LDH levels, slight raises CK-MB concentration range in group II. AST, ALT marker enzyme levels are higher in experimental rats with controls, troponin T values are clearly says that some cardio complications in group II compared to group I. Some other tests are done in heart tissues it is clearly observed that Ldh, Ck-Mb, Ast and Alt levels are decreased followed by antioxidant enzymes SOD, catalase but lipid peroxidation and protein carbonyls values are increased in nicotine induced rats and match up to normal rats (Table 2). Data presented in Table 3 GST levels are increases significantly and Gpx, GRd and GSH levels are decreased in group II nicotine induced rats and corporately control group I.Table 3

Table 1
Biochemical alteration of serum cardiac enzyme values in control and nicotine administrated gastrointestinal tumors rats.

Parameter	Groups	
	Group I Control rats	Group II Nicotine administrated gastrointestinal tumor rats
Body Weight (g)	402.24 ± 12.92	348.50 ± 11.24
Heart Weight (g)	0.96 ± 0.05	1.22 ± 0.14
Systolic blood pressure (mm Hg)	92.14 ± 30.16	74.80 ± 10.68
LDH (μ moles of NAD + liberated per minute per mg protein)	434.82 ± 32.78	460.32 ± 40.12
CK-MB (IU/L)	472.24 ± 30.12	484.44 ± 36.80
AST (μ moles of oxaloacetate liberated per minute per mg protein)	90.14 ± 7.68	104.22 ± 8.76
ALT (μ moles of pyruvate liberated per minute per mg protein)	34.68 ± 3.84	48.44 ± 4.68
Troponin T (n moles)	0.01 ± 0.001	3.12 ± 0.26

Values are expressed in all tables as Mean ± SD, significantly different is set ($P \leq 0.05$) to Duncan's Multiple Range (DMR) test, n = 12.

Table 2
Cardiac marker enzymes biochemical alterations in control and nicotine induced gastrointestinal tumors rats of heart tissue.

Parameter	Groups	
	Group I Control rats	Group II Nicotine administrated gastrointestinal tumor rats
Ldh (μ moles of NAD + liberated per minute per mg protein)	216.48 ± 16.92	190.88 ± 14.84
Ck-Mb (μ moles of phosphorus liberated per minute per mg protein)	16.60 ± 1.62	15.82 ± 1.38
Ast (μ moles of oxaloacetate liberated per minute per mg protein)	446.24 ± 36.80	416.72 ± 38.84
Alt (μ moles of pyruvate liberated per minute per mg protein)	382.54 ± 34.80	306.48 ± 30.22
SOD (units/mg protein)	10.06 ± 0.84	7.84 ± 0.68
Catalase ($\times 10^{-3}$ units/mg protein)	9.40 ± 0.82	8.36 ± 0.74
Lipid peroxidation (m moles/100 g wet tissue)	0.52 ± 0.05	0.84 ± 0.06
Protein carbonyls (n moles/mg protein)	1.50 ± 0.12	2.42 ± 0.24

Values are expressed in all tables as Mean ± SD, significantly different is set ($P \leq 0.05$) to Duncan's Multiple Range (DMR) test, n = 12.

Table 3
Heart tissue antioxidant enzyme levels in control rats and nicotine induced gastrointestinal tumors rats.

Parameter	Groups	
	Group I Control rats	Group II Nicotine administrated gastrointestinal tumor rats
GPx ($\times 10^{-2}$ units/mg protein)	0.72 ± 0.05	0.64 ± 0.04
GRd ($\times 10^{-2}$ units/mg protein)	4.54 ± 0.28	3.65 ± 0.26
GSH (m moles/100 g wet tissue)	114.80 ± 9.82	101.42 ± 8.96
GST (n moles of CDNB-GSH conjugate formed per min per mg protein)	7.80 ± 0.84	8.62 ± 0.74

Values are expressed in all tables as Mean ± SD, significantly different is set ($P \leq 0.05$) to Duncan's Multiple Range (DMR) test, n = 12.

4. Discussion

The present study paying attention taking place the modification in the (miRNA-1, -15b and -21) expression of heart after nicotine induced rats. We sought to reach the utmost injure of heart through minimum transience present experimentation and oxidative strain, cytotoxicity, fibrosis, inflammation, and the ordinary penaltities of other disclosure and cardiac hypertrophy represent (Pathak et al. 2015; Slezak et al., 2015). Freshly, similar report shows that modulate expression of miRNA levels in radiation effects menctioned (Chaudhry, 2014; Wang et al. 2015). Micro RNAs are in heart tissue in adaptation to stressors and important function in remodeling (Romaine et al. 2015). Here study; possible biomarkers i.e., miRNAs were of nicotine injure and restore. Selection of miRNAs to their recognized participation with cardiac hypertrophy (miRNA-1), fibrosis (miRNA-21), apoptosis (miRNA-15b, -21) Zhu and Fan (2012) and oxidative pressure (miRNA-15b, -21) (Simone et al., 2009). Old miRNAs be chosen support on its purpose in a lot of cardiovascular diseases plus its relationship with pathological development in the heart following exposure of irradiation (Slezak et al., 2013; 2014). The connection between the incidence of abdomen cancer and cigarette smoking (nicotine) has been deliberate since the 1950s. Cigarette smoking has been calculated as one of the key hazard feature for gastric cancer, which increases the amount of the disease by approximately 1.5- to 2.5-fold among recent smokers. Nicotine, the lively compound in cigarette smoke, has been recognized to be capable of promote gastric tumor growth and neovascularization. RO species are created increase rate in the myocardium with effect of nicotine. Cells of Myocardium include enzymes like, CK-MB, LDH, ALT, AST and structural proteins like troponins which are free in blood owing to necrosis and cellular dysfunction, consequence of oxidative pressure through MI. The hearts which have condensed the cardiac injure and so confine the emission of these enzymes and troponin T. Results without a doubt prove that cardiac injure. Further studies are need to interaction with nicotine and biological target molecules.

5. Conclusion

Nicotine induced gastrointestinal stromal tumors model rats the levels of miRNA-15b expression is decrease in group II but miRNA-21 expression values are increased in experimental rats (group II) with control rats (group I). Antioxidant enzyme (GST) levels are increases significantly and Gpx, GRd and GSH levels are decreased in group II nicotine induced rats and corporately control group I and some cardio marker enzyme levels were increases LDH levels, slight raises CK-MB concentration range it leads to coronary risk. More in strength studies are needed to interact of nicotine and miRNAs.

Ethical approval

This research was approved by Hanzhong 3201 Hospital, China, animal ethical committee, Approved No. HZ3201-13433.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Agaimy, A., Terracciano, L.M., Dirnhofer, S., Tornillo, L., Foerster, A., Hartmann, A., Bihl, M.P., 2009. V600E BRAF mutations are alternative early molecular events in a subset of KIT/PDGFRA wild-type gastrointestinal stromal tumours. J. Clin. Pathol. 62 (7), 613–616.

Agergaard, N., Jensen, P.T., 1982. Procedure for blood glutathione peroxidase determination in cattle and swineAnalyseprocedure til bestemmelse af glutathion peroxidase i kvæg-og svineblod. Acta Vet. Scand. 23 (4), 515–527.

Bartel, D.P., 2004. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116 (2), 281–297.

Chen, X.i., Ba, Y.i., Ma, L., Cai, X., Yin, Y., Wang, K., Guo, J., Zhang, Y., Chen, J., Guo, X., et al., 2008. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. Cell Res. 18 (10), 997–1006.

Chen, Y.J., Zhu, J.M., Wu, H., Fan, J., Zhou, J., Hu, J., et al., 2013. Circulating microRNAs as a fingerprint for liver cirrhosis. PLoS ONE 8, e66577.

Corless, C.L., Fletcher, J.A., Heinrich, M.C., 2004. Biology of gastrointestinal stromal tumors. J. Clin. Oncol. 22 (18), 3813–3825.

David, M., Richard, J.S., 1983. Glutathione reductase. In: Bergmeyer, H.U. (Ed.), Methods of Enzymatic Analysis. Verlag Chemie, Weinheim, Florida, pp. 258–265.

Ellman, G.L., 1959. Tissue sulfhydryl groups. Arch. Biochem. Biophys. 82 (1), 70–77.

Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249 (22), 7130–7139.

Heinrich, M.C., Corless, C.L., Duensing, A., McGreevey, L., Chen, C.J., Joseph, N., Singer, S., Griffith, D.J., Haley, A., Town, A., et al., 2003. PDGFRA activating mutations in gastrointestinal stromal tumors. Science 299, 708–710.

Hirota, S., Isozaki, K., Moriyama, Y., Hashimoto, K., Nishida, T., Ishiguro, S., Kawano, K., Hanada, M., Kurata, A., Takeda, M., et al., 1998. Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. Science 279, 577–580.

Hostein, I., Faur, N., Primois, C., Boury, F., Denard, J., Emile, J.-F., Bringuier, P.-P., Scoazec, J.-Y., Coindre, J.-M., 2010. BRAF mutation status in gastrointestinal stromal tumors. Am. J. Clin. Pathol. 133 (1), 141–148.

Kakkar, P., Das, B., Viswanathan, P.N., 1984. A modified spectrophotometric assay of superoxide dismutase. Indian J. Biochem. Biophys. 21, 130–132.

Katus, H.A., Remppis, A., Neumann, F.J., Scheffold, T., Diederich, K.W., Vinar, G., Noe, A., Matern, G., Kuebler, W., 1991. Diagnostic efficiency of troponin T measurements in acute myocardial infarction. Circulation 83 (3), 902–912.

King, J., 1965. The dehydrogenases or oxido reductase-Lactate dehydrogenase. In: Van, D. (Ed.), Practical Clinical Enzymology. Nostrand, London, pp. 83–93.

Lee, K.-F., Chen, Y.-C., Hsu, P.-C., Liu, I.Y., Wu, L.-H., 2014. MicroRNA expression profiling altered by variant dosage of radiation exposure. Biomed. Res. Int. 2014, 1–10.

Levine, R.L., Williams, J.A., Stadtman, E.R., Shacter, E., 1994. Carbonyl assays for determination of oxidatively modified proteins. Methods Enzymol. 233, 346–357.

Lewis, B.P., Burge, C.B., Bartel, D.P., 2005. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 120 (1), 15–20.

Li, D.F., Tian, J., Guo, X., Huang, L.M., Xu, Y., Wang, C.C., Wang, J.F., Ren, A.J., Yuan, W. J., Lin, L., 2012. Induction of microRNA-24 by HIF-1 protects against ischemic injury in rat cardiomyocytes. Physiol. Res. 61, 555–565.

Maehly, A.C., Chance, B., 1954. The assay of catalases and peroxidases. Methods Biochem. Anal. 1, 357–424.

Meder, B., Backes, C., Haas, J., Leidinger, P., Stähler, C., Großmann, T., Vogel, B., Frese, K., Giannitsis, E., Katus, H.A., et al., 2014. Influence of the confounding factors age and sex on microRNA profiles from peripheral blood. Clin. Chem. 60, 1200–1208.

Miettinen, M., Virolainen, M., 1995. Gastrointestinal stromal tumors value of CD34 antigen in their identification and separation from true leiomyomas and schwannomas. Am. J. Surg. Pathol. 19, 207–216.

Mitchell, P.S., Parkin, R.K., Kroh, E.M., Fritz, B.R., Wyman, S.K., Pogosova-Agadjanyan, E.L., Peterson, A., Noteboom, J., O'Briant, K.C., Allen, A., et al., 2008. Circulating microRNAs as stable blood-based markers for cancer detection. PNAS 105 (30), 10513–10518.

Reitman, S., Frankel, S., 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol. 28 (1), 56–63.

Rosalki, S.B., 1967. An improved procedure for serum creatine phosphokinase determination. J. Lab. Clin. Med. 69, 696–705.

Sushama Kumari, S., Varghese, A., Muraleedharan, D., Menon, V.P., 1990. Protective action of aspirin in experimental myocardial infarction induced by isoproterenol in rats and its effect on lipid peroxidation. Indian J. Exp. Biol. 28, 480–485.

Templin, T., Paul, S., Amundson, S.A., Young, E.F., Barker, C.A., Wolden, S.L., Smilenov, L.B., 2011. Radiation-induced micro-RNA expression changes in peripheral blood cells of radiotherapy patients. Int. J. Radiat. Oncol. Biol. Phys. 80 (2), 549–557.

van Rooij, E., Sutherland, L.B., Liu, N., Williams, A.H., McAnally, J., Gerard, R.D., Richardson, J.A., Olson, E.N., 2006. A signature pattern of stress-responsive microRNAs that can evoke cardiac hypertrophy and heart failure. Proc. Natl. Acad. Sci. U.S.A. 103 (48), 18255–18260.