

Aim of the study: Genistein, an isoflavonoid, plays roles in the inhibition of protein tyrosine kinase phosphorylation, induction of apoptosis, and cell differentiation in breast cancer. This study aims to induce cellular stress by exposing genistein to determine alterations of miRNA expression profiles in MCF-7 cells.

Material and methods: XTT assay and trypan blue dye exclusion assays were performed to examine the cytotoxic effects of genistein treatment. Expressions of miRNAs were quantified using Real-Time Online RT-PCR.

Results: The IC_{50} dose of genistein was 175 μ M in MCF-7 cell, line and the cytotoxic effect of genistein was detected after 48 hours. miR-23b was found to be up-regulated 56.69 fold following the treatment of genistein. It was found that miR-23b was up-regulated for MCF-7 breast cancer cells after genistein treatment.

Conclusions: Up-regulated expression of miR-23b might be a putative biomarker for use in the therapy of breast cancer patients. miR-23b up-regulation might be important in terms of response to genistein.

Key words: breast cancer, genistein, miRNA, MCF-7.

Contemp Oncol (Pozn) 2015; 19 (1): 32–35
DOI: 10.5114/wo.2014.44121

Genistein-induced mir-23b expression inhibits the growth of breast cancer cells

Cigir Biray Avci¹, Sunde Yilmaz Susluer¹, Hasan Onur Caglar², Tugce Balci¹, Duygu Aygunes¹, Yavuz Dodurga³, Cumhuri Gunduz¹

¹Department of Medical Biology, Medical Faculty, Ege University, Bornova, Izmir, Turkey

²Department of Stem Cell, Health Science Institute, Ege University, Bornova, Izmir, Turkey

³Department of Medical Biology, School of Medicine, Pamukkale University, Denizli, Turkey

Introduction

Breast cancer is the most common cancer type among women. After respiratory cancers, including lung and respiratory tract cancers, breast cancer is the second leading cause of death among women [1]. Incidence and mortality rates of breast cancer may differ depending on the age, ethnicity, and socioeconomic status of patients [2]. The presence or absence of oestrogen receptor (ER), progesterone receptor (PR), and *HER2/Neu* receptor determine the sub-molecular classification and progression of breast cancer [3]. miRNAs are non-coding small RNA molecules (17–24 nucleotide) that repress mRNA transcription when they bind to their target region (3' untranslated region). Regulation of expression profiles of miRNAs has a putative role in cancer development [4]. Decreasing expression of miRNA that suppress mRNA of oncogene, or increasing of expression of miRNA that suppress mRNA of tumour suppressor gene, can trigger the cancer process [4, 5]. It has been demonstrated that oncomirs such as miR-21, miR-27a, miR-155, and miR-145 exhibit different expression patterns between breast cancer cells and non-cancerous breast cells [6–8]. In addition to this difference, altered miRNA expression profile is detected differently among molecular sub-types of breast cancer [9]. Abnormal miRNA expression pattern can induce angiogenesis and metastasis in breast cancer tumours [10]. miRNA dysregulation in breast cancer is also associated with poor survival and poor therapeutic outcome [11, 12].

Genistein, an isoflavonoid, is a prime anti-cancer component of soybean, and it plays roles in the inhibition of protein tyrosine kinase phosphorylation, induction of apoptosis, and cell differentiation in breast cancer [13]. The interaction between genistein and oestrogen receptor signalling pathway in breast cancer has been well characterised. Genistein induces oestrogen-dependent cell growth and up-regulation of ER expression, thus it has a potential impact for hormone therapy [14, 15]. Genistein-mediated ER α expression is associated with histone modification changes and genistein re-sensitises ER α -negative breast cancer cells to tamoxifen [16]. Although it has been known that genistein affects chemotherapy agent efficiency and apoptosis, the effect of genistein on miRNA profiles is still unknown for breast cancer. This study aims to induce cellular stress by exposing genistein to determine the IC_{50} doses of treatment conditions in MCF-7 cells. This study also aims to evaluate the single effect in terms of miRNA expression levels.

Material and methods

Tumour cell line

Breast cancer cell line (MCF-7), which was purchased from ATCC, was used as a breast cancer model.

Cell culture

Breast cancer cell line (MCF-7) was cultured in RPMI-1640 medium supplemented with 100 IU/ml penicillin, 10 mg/ml streptomycin, 1% L-glutamine, and 10% heat-inactivated foetal bovine serum, at 37°C in a humidified 95% air 5% CO₂ atmosphere.

Treatment of genistein and cytotoxicity assay

MCF-7 cells were incubated at a density of 2×10^5 cells/ml of medium using 96-well plates for 24, 48, and 72 hours. Studied concentrations of genistein were 75 μ M, 100 μ M, 125 μ M, 150 μ M, 175 μ M, and 200 μ M. XTT assay and trypan blue dye exclusion assays were performed to examine the cytotoxic effect of IC₅₀ dose of genistein in the MCF-7 cell line. Formazan formation was quantified spectrophotometrically at 450 nm (reference wavelength 620 nm) with a microplate reader. Viability was calculated using the background-corrected absorbance. Cells without any treatment were taken as a control group.

Isolation of miRNA

miRNA was isolated from cells exposed to IC₅₀ dose of genistein and the control group. Isolation of miRNA and cDNA synthesis was performed using RT² qPCR-Grade miRNA Isolation Kit and RT² first Strand Kit, respectively, according to the manufacturers' instructions.

Relative quantification of miRNAs

Relative quantitation of 88 microRNAs (Table 1) was measured by using real-time online RT-PCR (LightCycler 480). SNORD48, SNORD47, SNORD44, U6 were used as human endogenous controls. Alterations in the miRNAs expressions of genistein were compared to the control group. Data analysis was evaluated by $\Delta\Delta$ CT method, "Light Cyler® 480 Quantification Software" program, and statistical analysis was evaluated with web-based RT² Profiler PCR Array Data Analysis.

Results

Cytotoxic effect of genistein on MCF-7 cells

Cells were incubated at a density of 2×10^5 cells/ml of medium using 96-well plates for 24, 48, and 72 hours. Studied concentrations of genistein were 75 μ M, 100 μ M, 125 μ M, 150 μ M, 175 μ M, and 200 μ M (Fig. 1). Untreated MCF-7 cells were considered as a control group. The IC₅₀ dose of genistein was 175 μ M and the cytotoxic effect of genistein was detected after 48 hours.

miR-23b is up-regulated miRNA by genistein in MCF-7 cell line

Alterations in the expressions of miRNAs were compared with genistein untreated MCF-7 cells. miRNA expression was detected 48 hours after genistein treatment. SNORD44, SNORD47, SNORD48, and U6 genes were

Table 1. Target and housekeeping miRNAs that were analysed for the genistein group. Expression analyses for 88 target miRNAs were performed by real-time PCR. Table 1 describes the sequences of miRNAs

miRNA	Sequence	miRNA	Sequence	miRNA	Sequence
hsa-miR-142-5p	CAUAAAGUAGAAAGCAUACU	hsa-miR-191	CAACGGAAUCCCAAAGACAGCUG	hsa-miR-320	AAAAGCUGGGUUGAGAGGGCGA
hsa-miR-16	UAGCAGCACGUAAAUAUUGGCG	hsa-miR-17	CAAAGUGCUUACAGUCAGGUAG	hsa-miR-374a	UUUAUAUACAACUCUGAUAAGUG
hsa-miR-142-3p	UGUAGUGUUUCCUACUUUAUGGA	hsa-miR-130a	CAGUGCAAUUGUAAAAGGGCAU	hsa-let-7e	UGAGGUAGGAGGUUGUAUGUU
hsa-miR-21	UAGCUUACAGACUGAUGUUGA	hsa-miR-20a	UAAAGUGCUUUAUGUCAGGUAG	hsa-miR-151-5p	UCGAGGAGCUCACAGUCUAGU
hsa-miR-15a	UAGCAGCACAUAAUGGUUUGUG	hsa-miR-27b	UUCACAGUGGCUAAGUUCUGC	hsa-miR-374b	AUAUAUAACAACUCGCUAAGUG
hsa-miR-29b	UAGCACCAUUGAAAUCAGUGUU	hsa-miR-26b	UUCAAGUAUUCAGGAUAGGU	hsa-miR-196b	UAGGUAGUUCCUGUUUGUGG
hsa-let-7a	UGAGGUAGUAGGUUGUAUAGUU	hsa-miR-146a	UGAGAUCUGAAUCCUAGGUGUU	hsa-miR-140-3p	UACCACAGGUGAAGACCACGG
hsa-miR-126	UCGUACCGUGAGUAAUAAUGCG	hsa-miR-200c	UAAUACUCGCGGGUAAUGAUGGA	hsa-miR-100	AACCCGUAAGUCCGAAUCUUGUG
hsa-miR-143	UGAGAUGAAGCACUGUAGCUC	hsa-miR-99a	AACCCGUAAGUCCGAAUCUUGUG	hsa-miR-103	AGCAGCAUUGUACAGGGCUAUGA
hsa-let-7b	UGAGGUAGUAGGUUGUGUGUU	hsa-miR-19a	UGUGCAAUUCUAGCAAACUGA	hsa-miR-96	UUUGGCACUAGCACAUUUUUGCU
hsa-miR-27a	UUCACAGUGGCUAAGUUCGCG	hsa-miR-23a	AUCAUUAUCCAGGGAAUUUCC	hsa-miR-302b	UAAUGUCUUCAUGUUUUAGUAG
hsa-let-7f	UGAGGUAGUAGUUGUAUAGUU	hsa-miR-30a	UGUAAACAUCUCCGACUGGAAG	hsa-miR-194	UGUAACAGCAACUCCUAGUGGA
hsa-miR-9	UCUUUGGUUAUCUAGCUGUAUGA	hsa-let-7i	UGAGGUAGUAGUUUGUCUGUU	hsa-miR-125a-5p	UCCUGAGACCCUUUAACCUGUGA
hsa-miR-26a	UUAAAGUAUCCAGGAUAGGCU	hsa-miR-93	CAAAGUCUGUUCGUCAGGUAG	hsa-miR-423-5p	UGAGGGCAGAGAGCAGACUUU
hsa-miR-24	UGGCUCAGUUCAGCAGGAACAG	hsa-let-7c	UGAGGUAGUAGUUUGUUGUU	hsa-miR-376c	AACAUAAGAGAAAUUCACAGU
hsa-miR-30e	UGUAAACAUCUCCUAGCUGGAAG	hsa-miR-106b	UAAAGUCUGACAGUCAGAU	hsa-miR-195	UAGCAGCACAGAAAUAUUGGC
hsa-miR-181a	AACAUCUCAAACGUCUGGUGAGU	hsa-miR-101	UACAGUACUGUGUAUACUGAA	hsa-miR-222	AGCUACAUCUGGCUACUGGGU
hsa-miR-29a	UAGCACCAUCUGAAAUCGGUUA	hsa-let-7g	UGAGGUAGUAGUUUGUACAGUU	hsa-miR-28-3p	CACUAGAUUGUGAGCUCUGGA
hsa-miR-124	UAAAGCCAGCGGUGAAUGCC	hsa-miR-425	AUUGACACAGUACUCCCGUUGA	hsa-miR-128a	UCACAGUAAACCGGUCUUUU
hsa-miR-144	UACAGUAUAGUAGUAGUACU	hsa-miR-15b	UAGCAGCACAUCAUGGUUUAC	hsa-miR-302c	UAAUGUCUUCAUGUUUCAGUGG
hsa-miR-30d	UGUAAAACAUCUCCGACUGGAAG	hsa-miR-28-5p	AAGGAGCUCACAGUCUAUUGAG	hsa-miR-423-3p	AGCUCGGUCUGAGGCCUUCAGU
hsa-miR-19b	UGUGCAAUCCUAGCAAACUGA	hsa-miR-18a	UAAAGGUCUUCUAGUCAGAUAG	hsa-miR-185	UGGAGAAAGAGCAGUUCUGA
hsa-miR-22	AAGCUGCCAGUUGAAGAACUGU	hsa-miR-25	CAUUGCACUUGUCUGGUCUGA	hsa-miR-30b	UGUAACAUCUUCACACUCAGCU
hsa-miR-122	UGGAGUGUACAAUGGUUUUG	hsa-miR-23b	AUCACAUUGCCAGGGAUUACC	hsa-miR-210	CUGUGCUGUGACAGCGGUCUGA
hsa-miR-150	UCUCCCAAUCCUUGUACAGAGU	hsa-miR-302a	UAAAGUCUCCUAGUUUUGGUGA	SNORD48	TAACTCTGAGTGTCTGCTGA
hsa-miR-32	UAUUGCACAUAUUAAGUUGCA	hsa-miR-186	CAAAGAAUUCUCCUUUGGGCU	SNORD47	CCGTCCATTTTGATTCTGAG
hsa-miR-155	UUAAUGCUAAUCUGUAUAGGGGU	hsa-miR-29c	UAGCACCAUUGGAAUUCGGUUA	SNORD44	GGTCTTAATTAGCTCTAACTGAC
hsa-miR-140-5p	CAGUGUUUUUACCUAUGGUAG	hsa-miR-7	UGGAAGACUAGUUAUUUUGUUU	U6	ATTGGAACGATACAGAGAAGATTAG
hsa-miR-125b	UCCUGAGACCCUAAUUGUGA	hsa-let-7d	AGAGGUAGUAGGUUGCAUAGUU	miRTC	ACACTAAGTACGTCGTATTAC
hsa-miR-141	UAAACACUGUCUGUAAAGAUGG	hsa-miR-30c	UGUAAACAUCUCCUACUCUCAGC	miRTC	ACACTAAGTACGTCGTATTAC
hsa-miR-92a	UAUUGCACAUUCUCCGCGUUG	hsa-miR-181b	AACAUAUUCUUGUUGUUGGUGU		
hsa-miR-424	CAGCAGCAAUUCUUGUUUGAA	hsa-miR-223	UGUCAGUUUGUCAAUACCCCA		

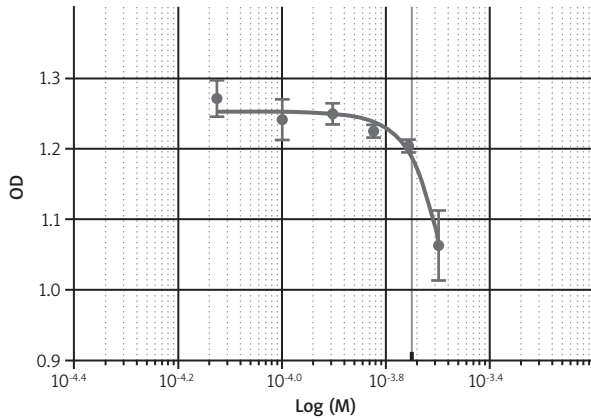


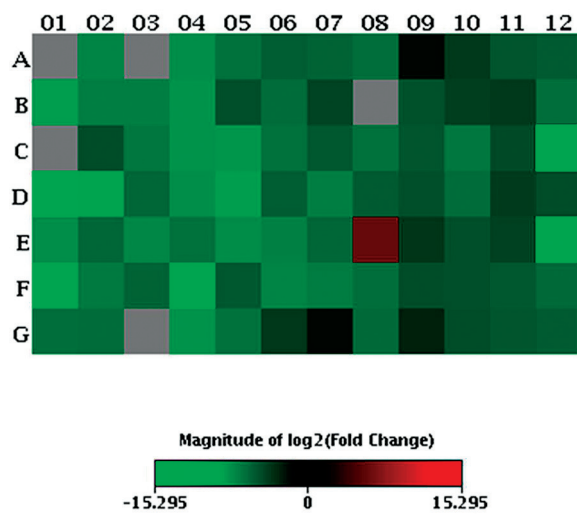
Fig. 1. Dose-dependent cytotoxicity of genistein. MCF-7 cells were treated with various concentrations of genistein. The studied concentrations of genistein were 75 μM, 100 μM, 125 μM, 150 μM, 175 μM, and 200 μM. The IC₅₀ dose of genistein was 175 μM

used for housekeeping miRNAs as the endogenous normalisation factor to define miRNA expression profiles of 88 miRNAs. miR-23b was found to be up-regulated 56.69 fold in the treatment of genistein compared to the control group of genistein untreated cells (Fig. 2).

Discussion

Several studies have reported that genistein, which is an isoflavonoid and is a prime anti-cancer component

of soybean, can affect miRNA expression levels [17–20]. miR-151, which has an oncogenic effect, is up-regulated in prostate cancer cell lines (PCa), and genistein treatment down-regulates the relative expression of miR-151 in PCa [21]. It is known that genistein induces expression of miR-574-3p, which has a tumour suppressor role, and this induction inhibits cell proliferation, migration, and invasion *in vitro* and *in vivo* for prostate cancers [22]. Zaman *et al.* showed that genistein decreases the expression of miR-23b-3p in A-498 renal cancer cell line [23]. Furthermore, suppression of miR-23b-3p increases the number of total apoptotic cells and decreases cell invasion [23]. Although it is known that genistein affects chemotherapy agent efficiency and apoptosis, the effect of genistein on miRNA profiles is still unknown for breast cancer. In this study, it was found that treatment condition, which was genistein, affected miRNA expressions in MCF-7 breast cancer cell line. The cytotoxic effects of the defined group were examined independently. Cells in the genistein group were treated with an IC₅₀ dose of genistein for three days. The cytotoxic effect of treatment group was observed after 48 hours. After the IC₅₀ dose of genistein was determined, miRNA qPCR array method was performed to detect regulation of miRNAs expressions in MCF-7 cell line. In this study, it was found that expression of miR-23b was up-regulated in the genistein treatment group. Majid *et al.* clearly showed that miR-23b is a methylation-silenced tumour suppressor in prostate cancer, and a high expression level of miR-23b is associated with higher survival rates in prostate cancer patients [24]. Stable ectopic expression of miR-23b in HCT-116 colon carcinoma cell line reduces migration, invasion, and resistance to anoikis [25]. *In vivo* tumour models, which are generated from miR-23b-expressing HCT 116 cells, show that miR-23b-expressing tumours are encapsulated, non-invasive, and have low growth rate [25]. miR-23b regulates colony morphology and increases epithelial characteristics in MCF-7 cells. It is observed that miR-23b enhances focal adhesion connections and provides less lamellipodia structure after transfection in MDA-MB-231 breast cancer cells [26]. miR-23b regulates cytoskeletal reorganization and reduces cell motility and invasion via the *PAK2* gene, which is a target for miR-23b in MCF-7 and MDA-MB-231 cells [26]. Furthermore, inhibition of miR-23b increases cell migration and metastasis for *in vivo* breast cancer models [26]. Because it is known that miR-23b has



	1	2	3	4	5	6	7	8	9	10	11	12
A	miR-142-5p	miR-16	miR-142-3p	miR-21	miR-15a	miR-29b	let-7a	miR-126	miR-143	let-7b	miR-27a	let-7f
	-62,47	-160,34	-62,47	-228,33	-88,95	-49,69	-58,28	-82,42	-4,13	-16,39	-40,09	-47,34
B	miR-9	miR-26a	miR-24	miR-30e	miR-181a	miR-29a	miR-124	miR-144	miR-30d	miR-19b	miR-22	miR-122
	-360,79	-132,06	-131,14	-267,8	-33,47	-76,37	-24,17	-62,47	-34,9	-19,49	-17,57	-83
C	miR-150	miR-32	miR-155	miR-140-5p	miR-125b	miR-141	miR-92a	miR-424	miR-191	miR-17	miR-130a	miR-20a
	-62,47	-30,38	-105,05	-273,42	-279,17	-92,73	-41,5	-86,52	-40,36	-106,52	-28,74	-40202,57
D	miR-27b	miR-26b	miR-146a	miR-200c	miR-99a	miR-19a	miR-23a	miR-30a	let-7i	miR-93	let-7c	miR-106b
	-1108,97	-489,44	-65,57	-217,52	-373,51	-50,39	-137,66	-47,34	-33,94	-73,26	-17,81	-31,23
E	miR-101	let-7g	miR-425	miR-15b	miR-28-5p	miR-18a	miR-25	miR-23b	miR-302a	miR-186	miR-29c	miR-7
	-217,52	-62,03	-168,31	-92,73	-197,4	-150,64	-65,12	56,69	-14,07	-31,89	-21,63	-6912,54
F	let-7d	miR-30c	miR-181b	miR-223	miR-320a	miR-374a	let-7e	miR-151-5p	miR-374b	miR-196b	miR-140-3p	miR-100
	-590,18	-118,19	-59,1	-716,59	-42,67	-153,81	-123,21	-85,33	-29,96	-32,56	-42,08	-69,31
G	miR-103	miR-96	miR-302b	miR-194	miR-125a-5p	miR-423-5p	miR-376c	miR-195	miR-222	miR-28-3p	miR-128	miR-302c
	-80,17	-75,85	-62,47	-264,11	-92,09	-15,94	-4,18	-71,26	-7,75	-30,38	-38,72	-47,34

Fig. 2. miRNA expression profiles after treatment. For the genistein group, miR-23b was up-regulated 56.69 fold after treatment. miRNA expression visualization about log₂ (Fold Change) associated with genistein, compared with control

a tumour suppressor role for metastasis of breast cancer cells, miR-23b up-regulation might be important in terms of response to genistein.

In conclusion, up-regulated expression of miR-23b might be a putative biomarker for use in the therapy of breast cancer patients.

The authors declare no conflict of interest.

This study was supported by Ege University Medical Faculty Research Project Subcommittee (Grant number:2008/TIP/021).

References

- Siegel R, Ward E, Brawley O, Jemal A. Cancer statistics, 2011: the impact of eliminating socioeconomic and racial disparities on premature cancer deaths. *CA Cancer J Clin* 2011; 61: 212-36.
- DeSantis C, Siegel R, Bandi P, Jemal A. Breast cancer statistics, 2011. *CA Cancer J Clin* 2011; 61: 409-18.
- Sorlie T, Perou CM, Tibshirani R, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci U S A* 2001; 98: 10869-74.
- Hammond SM. RNAi, microRNAs, and human disease. *Cancer Chemother Pharmacol* 2006; 58 Suppl 1: s63-8.
- Iorio MV, Casalini P, Piovan C, Braccioli L, Tagliabue E. Breast cancer and microRNAs: therapeutic impact. *Breast* 2011; 20 Suppl 3: S63-70.
- Iorio MV, Ferracin M, Liu CG, et al. MicroRNA gene expression deregulation in human breast cancer. *Cancer Res* 2005; 65: 7065-70.
- Guttilla IK, White BA. Coordinate regulation of FOXO1 by miR-27a, miR-96, and miR-182 in breast cancer cells. *J Biol Chem* 2009; 284: 23204-16.
- Li L, Xiao B, Tong H, Xie F, Zhang Z, Xiao GG. Regulation of breast cancer tumorigenesis and metastasis by miRNAs. *Expert Rev Proteomics* 2012; 9: 615-25.
- Lowery AJ, Miller N, Devaney A, et al. MicroRNA signatures predict oestrogen receptor, progesterone receptor and HER2/neu receptor status in breast cancer. *Breast Cancer Res* 2009; 11: R27.
- Harquail J, Benzina S, Robichaud GA. MicroRNAs and breast cancer malignancy: an overview of miRNA-regulated cancer processes leading to metastasis. *Cancer Biomark* 2012; 11: 269-80.
- Lyng MB, Laenkholm AV, Sokilde R, Gravgaard KH, Litman T, Ditzel HJ. Global microRNA expression profiling of high-risk ER+ breast cancers from patients receiving adjuvant tamoxifen mono-therapy: a DBCG study. *PLoS One* 2012; 7: e36170.
- Rothe F, Ignatiadis M, Chaboteaux C, et al. Global microRNA expression profiling identifies MiR-210 associated with tumor proliferation, invasion and poor clinical outcome in breast cancer. *PLoS One* 2011; 6: e20980.
- Orlando L, Schiavone P, Cinieri S. Genistein: the future of prevention and treatment of breast cancer? *Cancer Biol Ther* 2011; 11: 918-20.
- Beck V, Unterrieder E, Krenn L, Kubelka W, Jungbauer A. Comparison of hormonal activity (estrogen, androgen and progestin) of standardized plant extracts for large scale use in hormone replacement therapy. *J Steroid Biochem Mol Biol* 2003; 84: 259-68.
- van Duursen MB, Nijmeijer SM, de Morree ES, de Jong PC, van den Berg M. Genistein induces breast cancer-associated aromatase and stimulates estrogen-dependent tumor cell growth in vitro breast cancer model. *Toxicology* 2011; 289: 67-73.
- Li Y, Meeran SM, Patel SN, Chen H, Hardy TM, Tollefsbol TO. Epigenetic reactivation of estrogen receptor-alpha (ERalpha) by genistein enhances hormonal therapy sensitivity in ERalpha-negative breast cancer. *Mol Cancer* 2013; 12: 9.
- Parker LP, Taylor DD, Kesterson J, Metzinger DS, Gercel-Taylor C. Modulation of microRNA associated with ovarian cancer cells by genistein. *Eur J Gynaecol Oncol* 2009; 30: 616-21.
- Li Y, VandenBoom TG, 2nd, Kong D, Wang Z, Ali S, Philip PA, Sarkar FH. Up-regulation of miR-200 and let-7 by natural agents leads to the reversal of epithelial-to-mesenchymal transition in gemcitabine-resistant pancreatic cancer cells. *Cancer Res* 2009; 69: 6704-12.
- Li Y, Vandenboom TG, 2nd, Wang Z, Kong D, Ali S, Philip PA, Sarkar FH. miR-146a suppresses invasion of pancreatic cancer cells. *Cancer Res* 2010; 70: 1486-95.
- Sun Q, Cong R, Yan H, et al. Genistein inhibits growth of human uveal melanoma cells and affects microRNA-27a and target gene expression. *Oncol Rep* 2009; 22: 563-7.
- Chiyomaru T, Yamamura S, Zaman MS, et al. Genistein suppresses prostate cancer growth through inhibition of oncogenic microRNA-151. *PLoS One* 2012; 7: e43812.
- Chiyomaru T, Yamamura S, Fukuhara S, et al. Genistein up-regulates tumor suppressor microRNA-574-3p in prostate cancer. *PLoS One* 2013; 8: e58929.
- Zaman MS, Thamminana S, Shahryari V, et al. Inhibition of PTEN gene expression by oncogenic miR-23b-3p in renal cancer. *PLoS One* 2012; 7: e50203.
- Majid S, Dar AA, Saini S, et al. miR-23b represses proto-oncogene Src kinase and functions as methylation-silenced tumor suppressor with diagnostic and prognostic significance in prostate cancer. *Cancer Res* 2012; 72: 6435-46.
- Zhang H, Hao Y, Yang J, Zhou Y, Li J, Yin S, Sun C, Ma M, Huang Y, Xi JJ. Genome-wide functional screening of miR-23b as a pleiotropic modulator suppressing cancer metastasis. *Nat Commun* 2011; 2: 554.
- Pellegrino L, Stebbing J, Braga VM, et al. miR-23b regulates cytoskeletal remodeling, motility and metastasis by directly targeting multiple transcripts. *Nucleic Acids Res* 2013; 41: 5400-12.

Address for correspondence

Assistant Prof. Dr. **Yavuz Dodurga**, PhD
Department of Medical Biology
Pamukkale University
Denizli, Turkey
tel. +90 258 296 25 34
e-mail: yavuzdodurga@gmail.com

Submitted: 31.03.2014

Accepted: 30.06.2014