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Comparison of MED 12 gene mutation and microRNA-124 expression in leiomyoma and myometrium of Turkish patients

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ABSTRACT

Objectives: It is believed that there are still unclear areas in the formation mechanism of leiomyomas. In our study, it was aimed to investigate the formation mechanisms of leiomyomas due to local MED 12 gene exon 2 mutation and local microRNA-124 expression in a Turkish population.

Material and methods: Thirty patients who underwent hysterectomy for leiomyoma uteri at Gaziantep University between January 2013 and January 2016 were included in our study. In the pathology specimens of these patients, the patient's myometrium tissue and her own leiomyoma tissue were analysed via quantitative Realtime PCR in association with MED 12 exon 2 mutation and microRNA-124 expression.

Results: The average age of the 30 patients included in our study is 46.67 ± 5.42 and 13 patients had single leiomyoma; 17 patients had more than one leiomyoma. There were significantly higher c.130G>T (p.G44C) mutation and c.131G>A (p.G44A) mutation of MED 12 gene exon in leiomyoma tissues than healthy myometrium tissues of same patients. There was a 3.7-fold decrease in the expression of microRNA-124 in leiomyoma tissues compared to intact eutopic myometrium tissues, but this difference was not statistically significant.

Conclusions: In recent studies, it has been suggested that MED 12 gene may play an active role in the formation of fibroids. MED12 and β-catenin / Wnt pathway were emphasized, and alternative genetic pathways are sought in fibroid formation. Also, tumour suppressor and oncogenesis effects of microRNAs have been demonstrated in many different studies. Since it is involved in the Wnt pathway, microRNA-124 has been blamed by some previous studies for the formation of fibroids. This study demonstrates that MED12 exon 2 mutations and probably microRNA-124 gene expressions might contribute to uterine leiomyoma pathology.

Key words: anormal uterin bleeding; leiomyoma; MED 12; microRNA-124,

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INTRODUCTION

Also known as fibroids, uterine leiomyomas are the most common benign gynaecological tumours in women in reproductive age. Averagely, between 10% and 30% of cases cause abnormal uterine bleeding, recurrent pregnancy loss, pelvic pain, preterm birth and infertility [1, 2]. Nulliparity, early menarche, late menopause and obesity enlarge lesion size. However, lesion shrinking after menopause makes us consider that oestrogen and progesterone have a significant role in leiomyoma pathogenesis [3]. Cellular and molecular pathogenesis of common leiomyomas has not been revealed yet. It has been suggested that each leiomyoma stems from a mutant myometrial smooth muscle stem cell [4].

In some studies that research genetic factors underlying uterine leiomyomas, it is claimed that, in 40–50% of uterine myomas, certain gene regulations including 7q deletion and some different chromosomal aberrations such as 12q15 and 6p21 are observed [5, 6]. In addition to chromosomal change, Makinen et. al. [7], found that, in 71% of uterine leiomyoma cases, tissues had mutation in mediator complex subunit 12 (MED12) exon 2. MED12 is situated on chromosome sub-band Xq13 and consists of 45 exons. However,

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it is observed that leiomyoma mutations are only existent in 36.–44. codons in exon 2 [8, 9]. The prevalence of MED12 mutation is studied with different ethnic groups and it is found that its prevalence ranges from 50% to 80%, depending on ethnic group [8, 10, 11]. MED12 mutation is also seen in leiomyosarcoma (52%) and uterine smooth muscle tumour of uncertain malignant potential (STUMPs) (8%) [12].

In some other studies, it is illustrated that microRNAs regulate gene expressions by degrading or repressing messenger RNAs [13–15]. A mature microRNAs complex, combined collaterally with target gene sequences, results in gene expression degradations or gene expression decrease [16]. It is also known that microRNAs act as oncogenesis and tumour suppressor gene [17]. Similarly, in leiomyoma cases, microRNA expressions at different levels and microRNA deregulation are reported. Therefore, they are mostly associated with leiomyoma pathogenesis by many researchers [18]. Although many microRNAs are identified to have a role in leiomyoma formation, microRNA-124, which, like MED12, is situated in Wnt pathway, has not yet been found to have any impact on leiomyoma formation [19].

In these contexts, in this study which consists of a population of some Turkish patients, it is aimed to compare microRNA-124 expression differences and MED 12 gene exon 2 region mutations between specimens of leiomyoma tissues and myometrium tissues of the patients.

MATERIAL AND METHODS

In this retrospective study, paraffin blocks were obtained from total abdominal hysterectomy specimens of patients with a diagnosis of uterine leiomyoma, who visited Gaziantep University, Medical School, Department of Obstetrics and Gynaecology between January 2013 and January 2016. Inclusion criteria were total abdominal hysterectomy due to leiomyoma resulting in irregular bleeding, pelvic pain and sensation of pelvic fullness without the diagnosis that have been reported among the exclusion criteria. Exclusion criteria were patients with other gynaecologic diseases (endometriosis, premalignant or malignant gynaecological disease), systemic disease or other system malignancies. Ethics committee approval for the study was obtained from the Clinical Research Ethics Committee of Gaziantep University (No: 255, 26th September 2016). The consent form was obtained from the patients and tissue samples were transferred to Department of Molecular Medicine, Istanbul University Aziz Sancar Institute of Experimental Medicine (ASDETAE). Experimental stages of the study were carried out in that laboratory. Demographic characteristics and histopathological diagnosis of the patients were all recorded. DNA isolation, exon 2 region of MED12 gene with Polimerase chain reaction (PCR) and Sequencing method and microRNA-124 levels of leiomyoma tissues (leiomyoma group) and intact eutopic myometrium tissues of the same patients (control group) were studied. Paraffin embedded uterine tissue samples were divided into two groups (Group 1: Leiomyoma group, Group 2: Control group).

DNA and Total RNA isolation from paraffin tissue

The pieces cut from the paraffin block with microtome were treated with xylol and the paraffin was dissolved to provide tissue cleaning. DNA and Total RNA was isolated from 30 leiomyoma and 30 intact eutopic myometrium tissues with the QIAzol Lysis Reagent®QIAGEN from ~ 30 mg sections of each sample to the different protocol [20, 21].

Amplification and Sequencing of Exon 2 region of MED12 gene by PCR method

Primary sequences used to amplify the PCR fragment of Exon 2 region of MED12 gene are as follows: Forward: 5′-CCC CTT CCC CTA AGG AAA AA-3′, Reverse: 5′-ATG CTC ATC CCC AGA GAC AG-3′. Thermal cycler (T100™; BioRad, CA, USA) was used for PCR amplifications. PCR amplification was performed in a total volume of 25 µL with 1 µL of 150–200 ng DNA, 1 µL of forward and reverse primers (50 pmol/µL), 5 µL of dNTPs (1 mM), 1.5 µL of 10X Taq tampon, 1.5 µL of MgCl2 (25 mM), 0.3 µL of Taq DNA polymerase (5 u/µL) (GeneMarkGMbiolab Co., Ltd. Taichung, Taiwan) and 14.7 µL of distilled H_2O. The PCR mixture was incubated for five minutes at 95°C, followed by 30 cycles of 45 seconds at 94°C, 45 seconds at 59°C and 45 seconds at 72°C and a final step at 72°C for five minutes. The synthesized PCR products were separated by agarose gel electrophoresis and it was checked whether the DNA fragment of the desired size was amplified. PCR products were purified, and DNA sequencing was performed to the Sanger sequencing method which was studied by Dogan et al. [22].

microRNA-124 expression analysis

Total RNAs were diluted to 1µg/5µL and QIAGEN miScript®microRNA-124 detection kit was used for complementary DNA (cDNA) synthesis. The PCR was carried out with a total volume of 20 µL containing 5 µL of Total RNA, 4 µL of 5 × miScriptHiFlex Buffer, 2 µL of 10 × miScript nucleic mixture, 2 µL of reverse transcriptase and 7 µL of RNase free water. The reactions were subjected to 25°C for 10 min, 37°C for 60 min, 95°C for 5 min, and 4°C hold. For quantitative real-time PCR (qPCR), the U6 gene was used as reference gene for the RNA expression detection. After cDNA synthesis, Real-Time PCR analysis was performed with QIAGEN miScript SYBR ® Green PCR kit. The qPCR reactions were carried out with a total volume of 12.5 µL containing 6.25 µL of 2x Quantitech SYBR Green PCR Master Mix, 1.25 µL of 10x miScript Universal Primer, 1.25 µL of 10x miScript Primer Assay, 1,25 µL of cDNA, and 2.5 µL of RNase free

water. The qPCR reactions were subjected to hot start at 95°C for 15 min, followed by 40 cycles of denaturation at 94°C for 15 s, annealing 55°C for 30 s, and extension at 70°C for 30 s using the real-time detection system. Real-time PCR was performed twice for each sample. CT was obtained as a real-time PCR result for each repetition of each gene. The mean CT value was calculated by averaging the two results. The expression of genes was quantified by measuring the cycle threshold (Ct) values and normalized using the $2^{-\Delta\Delta CT}$ method relative to the U6 RNA [23].

Statistical analysis

Statistical analyses were performed using the IBM Statistical Package for Social Sciences 22nd version. The Kolmogorov-Smirnov, Student's t test and Mann–Whitney U tests were used in the statistical analysis of the data. The value of $p < 0.05$ was considered as statistically significant.

RESULTS

There were a total 60 tissue samples belonging to 30 patients that were included in the study. Samples taken from the leiomyoma tissues of the patients were compared with the samples taken from the patients' own eutopic myometrium tissues. The mean age of the patients included in the study was 46.67 ± 5.42 (min: 38, max: 68). The mean size of leiomyomas was 6.17 ± 4.74 cm (min: 1 cm, max: 23 cm) and the mean number of leiomyomas was 4.90 ± 5.62 (min: 1, max: 25). The levels of white blood cells (WBC), hemoglobin, platelets, glucose, blood urea nitrogen (BUN), creatinine, aspartate transaminase (AST), and alanine transaminase (ALT) of the patients were 7.43 ± 1.85 (10^6/mL) (min: 3, max: 11), 11.53 ± 2.42 (gr/dL) (min: 4, max: 16), 36.23 ± 6.35 (%) (min: 15, max: 46), 304.93 ± 75.57 (10^6/mL) (min: 166, max: 473), 111.27 ± 55.95 (mg/dL) (min: 75, max: 377), 27.23 ± 7.68 (mg/dL) (min: 15, max: 48), 0.9 ± 0.40 (mg/dL) (min: 0, max: 2), 20.17 ± 10.76 (U/L) (min: 10, max: 60), and 17.53 ± 9.00 (U/L) (min: 7, max: 41); respectively. Thirteen pateitns included in the study had one leiomyoma (43.3%) whereas 17 had more than one leiomyoma (56.7%). Two patients had only subserous leiomyomas, three had only intraligamentary leiomyomas, four had only submucous leiomyomas, and 11 had only intramural leiomyomas. Other patients had leiomyomas located in two or three different regions.

DNA sequences obtained from leiomyoma and myometrium tissues of 30 patients were analyzed by Sanger sequencing method. Exon 2 region of MED12 gene c.130G>T, p.G44C mutation was found in 14 leiomyomas (46.6%) ($p < 0.001$) (Fig. 1), and c.131G>A (p.G44A) mutation was observed in 6 of leiomyoma tissues ($p = 0.01$) (Fig. 2). No such mutation was observed in healthy myometrium

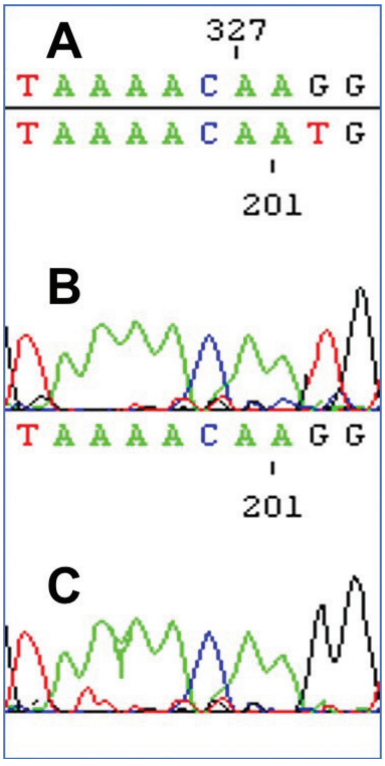

Figure 1. MED12 gene exon 2 c.130G>T, p.G44C mutation; **A.** MED12 mRNA (NM_005120.2) reference sequence; **B.** Mutant sequence in leiomyoma tissue (c.130G>T, p.G44C); **C.** Normal sequence of myometrium tissue

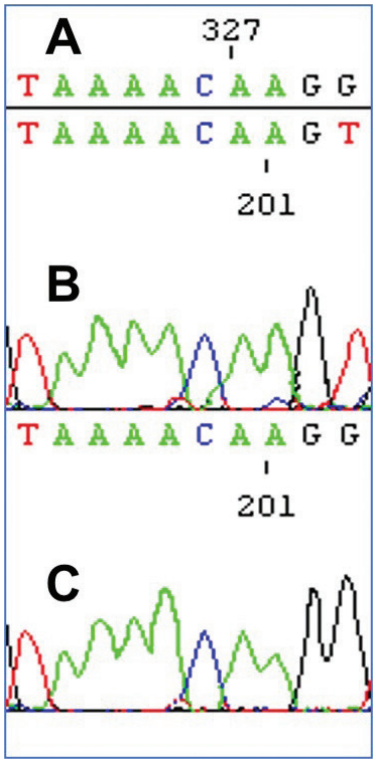

Figure 2. MED12 gene exon 2 c.131G>A, p.G44A mutation; **A.** MED12 mRNA (NM_005120.2) reference sequence; **B.** Mutant sequence in leiomyoma tissue (c.131G>A, p.G44A); **C.** Normal sequence of myometrium tissue

tissues of same patients. According to the results of our study, c.130G>T, p.G44C mutation and c.131G>A (p.G44A) mutation of MED 12 gene exon 2 were found significantly higher in leiomyoma tissue.

The expression levels of microRNA-124 were calculated according to the $2^{-\Delta\Delta CT}$ method (Tab. 1). There was a 3.7-fold decrease in the expression of microRNA-124 in leiomyoma tissues compared to intact eutopic myometrium tissues, but this difference was not statistically significant (p = 0.109) (Fig. 3).

There was no significant correlation between miR-124 expression levels and the MED12c.130G>T, p.G44C mutation in tissues with the mutation (p = 0.125).

DISCUSSION

Uterine leiomyomas are the most common benign mesenchymal neoplasm in women [8, 9]. Along with underlying etiological factors, genetic factors that might lead to leiomyoma formation have recently attracted many researchers. Therefore, there are now many studies regarding this subject.

In recent studies, the function of MED12 mutation in leiomyoma pathogenesis has been studied. For example, Di Tomasso et al. analysed MED12 mutation on leiomyoma, myometrium and pseudocapsule and reported both distinguished mutation profiles and IGF-2 levels between leiomyoma and pseudocapsule [24, 25]. It is also demonstrated that MED12 mutation disrupts the relation between MED12 and Cyclin C, CDK8/19, and that it also harms mediator-associated CDK kinase activity [26]. In some other studies, the relation between MED12 and β-catenin/Wnt pathway is revealed [7, 27]. Wnt pathway's selective inactivation decreases normal uterine myometrial cell formation and supports its conversion into a small but fart-rich uterus. This shows us the significance of Wnt signal for normal uterus development [28].

Table 1. Expression values for microRNA-124

	Leiomyoma	Myometrium
ΔCT	–0.938	–2.835
ΔΔCT	1.897	
$2^{-\Delta\Delta CT}$	0.27	
Fold	–3.7	
p	0.109	

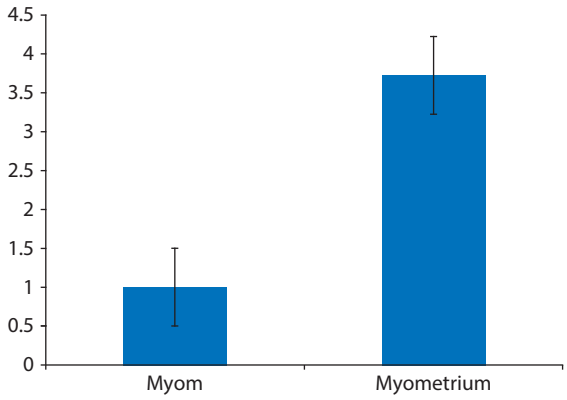

Figure 3. The microRNA-124 expression in leiomyoma and myometrium tissues

MED 12 is a 25-subunit protein complex that ensures cell development and durability by regulating RNA polymerase 2 in association with CDK8 [29]. The MED12 mutation, which is effective in Wnt pathway, is seen uterine leiomyomas (30–52%), leiomyosarcoma (14-20%) and unclear smooth muscle tumours with a potential malignity (8%) [12, 30, 31]. MED12 is situated on chromosome sub-band Xq13 and consists of 45 exons. However, it is observed that leiomyoma mutations are only existent in 36.–44. codons in exon 2 [8, 9]. The prevalence of MED12 mutation ranges from 50% to 80%, depending on ethnic group [8, 10]. Je et al., examined 1862 tumour tissues that include carcinoma, leukaemia and stromal tumours. They reported that MED12 mutation was seen in 52.2% of uterine leiomyoma cases and 0.3% of colon carcinomas [12]. In these contexts, in our study, cDNA sequence, which is obtained from leiomyoma and myometrium tissues of 30 patients via Sanger sequencing method, is analysed. Findings of the study illustrate that 67% of the patients have MED12 exon 2 mutations. In 14 leiomyoma tissues, c.130G>T (p.G44C) mutation was seen in exon 2 region of MED12 gene (70%). On the other hand, in six cases, c.131G>A (p.G44A) mutation was observed (30%).

It is demonstrated that MicroRNAs can be used as biomarkers for many malign and benign diseases [32]. MicroRNAs are key molecules in post-transcriptional regulation of gene expression and aberrant gene expression-oriented microRNA-124 expression alterations. Irregular microRNA expression is associated with tumuorigenesis, tumour progression, fibrosis, and tumour immunity. Similarly, microRNA-124 expression is observed in various sarcoma cases including GIST [33] and uterine leiomyoma cases [34]. Some microRNAs are expressed at different ratios in leiomyoma cases seen in different races [35]. These racial differences regarding microRNA expression play a significant role determining biomarkers, molecular pathogenesis and potential leiomyoma treatments.

Some inhibitors of Wnt signal pathway are run by microRNAs, and microRNA-124 is one of them [36]. MicroRNA-124's role in tumour progression, motility and angiogenesis has been studied and it is identified as a tumour suppressor microRNA [37, 38]. It is also demonstrated that microRNA-124 suppresses some other tumour functions

such as proliferation, activation, invasion, metastasis, and migration. In colorectal carcinomas, breast cancers, osteosarcoma, lung cancers and gastric cancers, abnormal expression of microRNA-124 is observed, as well [39–43]. On the other hand, microRNA-124 expression is reported to be decreasing in ovarian cancer [38], cervical cancer [44] and endometrial cancer [45]. There are some other studies illustrating that it influences proinflammatory cytokine leiomyomas [46–49]. For example, it is reported that microRNA-124 regulates cholinergic anti-inflammatory effect by inhibiting LPS-induced proinflammatory cytokines [50–53]. In the present study, it is also found that leiomyoma tissue has decreased 3.7 times as much as a healthy myometrium tissue since it has microRNA-124 expression. However, this difference is not determined to be statistically significant ($p = 0.109$). There are several studies found the relation between Wnt pathway and miR-124 expression. Hu et al. [54], found that miR-124 had lower expression levels in nasopharyngeal carcinoma compared to normal nasopharyngeal cells. They indicated that miR-124 suppresses of proliferation and invasion of nasopharyngeal carcinoma cells targeting Capn4 and the components of the Wnt/β-catenin signaling pathway [54]. Another study showed that overexpression of miR-124 suppressed the activity of non-canonical Wnt signaling, downstream of ROR2 and they suggested that miR-124 expression might inhibits osteosarcoma metastasis [55]. Furthermore, miR-124 might be a strategy for multiple-drug resistance. Long et al., reported that Wnt signaling is triggered by binding of Wnt ligands to Frizzled receptor proteins and this receptor/protein kinase C (PKC) signaling is responsible for the elevation of P-glycoprotein which is associated with multiple-drug resistance and cancer cell survival. They found miR-124 targeted to Frizzled receptor and ad significant inhibitory effects on P-glycoprotein expression. Thus, they suggested that miR-124 expression might be new therapy strategy to overcome P-glycoprotein mediated multiple drug resistance [56].

CONCLUSIONS

This study is unique in that it is the first study in Turkey that has been carried out about uterine leiomyoma cases regarding somatic mutations on exon 2 of MED12 complex and microRNA-124 gene expressions.

Age range of patients included in our study was between 38 and 68, so at least one patient was postmenopausal and expression of mir124 might be dependent on steroidal hormones, ideally all tested sample should be either pre-menopausal or post-menopausal for a homogeneous conclusion. This is clearly a limitation of our study. Also, the relatively small sample size may be another limitation.

This study demonstrates that both MED12 exon 2 mutations and microRNA-124 gene expressions might contribute to uterine leiomyoma pathology. When the prevalence of uterine leiomyomas and its impact on the quality of life are taken into consideration, it can be argued that, in the future, MED12 and microRNA-124 will be main focus of potential treatment strategies.

Ethics committee approval

Ethics committee approval was received for this study from the ethics committee of Gaziantep University.

Financial disclosure

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Conflict of interest

No conflict of interest was declared by the authors.

REFERENCES

1. Stewart EA. Uterine fibroids. Lancet. 2001; 357: 293–298.
2. Marshall LM, Spiegelman D, Goldman MB, et al. A prospective study of reproductive factors and oral contraceptive use in relation to the risk of uterine leiomyomata. Fertil Steril. 1998; 70(3): 432–439, doi: 10.1016/s0015-0282(98)00208-8, indexed in Pubmed: 9757871.
3. Jacobson GF, Shaber RE, Armstrong MA, et al. Hysterectomy rates for benign indications. Obstet Gynecol. 2006; 107(6): 1278–1283, doi: 10.1097/01.AOG.0000210640.86628.ff, indexed in Pubmed: 16738152.
4. De La Cruz MS, Buchanan EM. Uterine Fibroids: Diagnosis and Treatment. Am Fam Physician. 2017; 95(2): 100–107.
5. Kurose K, Mine N, Doi D, et al. Novel gene fusion ofCOX6C at 8q22-23 toHMGIC at 12q15 in a uterine leiomyoma. Genes, Chromosomes and Cancer. 2000; 27(3): 303–307, doi: 10.1002/(sici)1098-2264(200003)27:3<303::aid-gcc11>3.0.co;2-3.
6. Laganà AS, Vergara D, Favilli A, et al. Epigenetic and genetic landscape of uterine leiomyomas: a current view over a common gynecological disease. Arch Gynecol Obstet. 2017; 296(5): 855–867, doi: 10.1007/s00404-017-4515-5, indexed in Pubmed: 28875276.
7. Mäkinen N, Mehine M, Tolvanen J, et al. MED12, the mediator complex subunit 12 gene, is mutated at high frequency in uterine leiomyomas. Science. 2011; 334(6053): 252–255, doi: 10.1126/science.1208930, indexed in Pubmed: 21868628.
8. Markowski DN, Bartnitzke S, Loning T, et al. MED12 mutations in uterine fibroids the irrelations hiptocytogenetic subgroups. International Journal of Cancer. 2012.
9. Mello JBH, Barros-Filho MC, Abreu FB, et al. MicroRNAs involved in the HMGA2 deregulation and its co-occurrence with MED12 mutation in uterine leiomyoma. Mol Hum Reprod. 2018; 24(11): 556–563, doi: 10.1093/molehr/gay037, indexed in Pubmed: 30376129.
10. Hashimoto K, Azuma C, Kamiura S, et al. Clonal determination of uterine leiomyomas by analyzing differential inactivation of the X-chromosome-linked phosphoglycerokinase gene. Gynecol Obstet Invest. 1995; 40(3): 204–208, doi: 10.1159/000292336, indexed in Pubmed: 8529956.
11. Flynn M, Jamison M, Datta S, et al. Health care resource use for uterine fibroid tumors in the United States. Am J Obstet Gynecol. 2006; 195(4): 955–964, doi: 10.1016/j.ajog.2006.02.020, indexed in Pubmed: 16723104.
12. Je EMi, Kim MR, Min KiO, et al. Mutational analysis of MED12 exon 2 in uterine leiomyoma and other common tumors. Int J Cancer. 2012; 131(6): E1044–E1047, doi: 10.1002/ijc.27610, indexed in Pubmed: 22532225.
13. Kittelmann S, McGregor AP. Modulation and Evolution of Animal Development through microRNA Regulation of Gene Expression. Genes (Basel). 2019; 10(4), doi: 10.3390/genes10040321, indexed in Pubmed: 31027314.
14. Alsaadoni H, Çaykara B, Pençe S, et al. The expression levels of miR-655--3p, miR127-5p, miR-369-3p, miR-544a in gastric cancer. Turkish Journal of Biochemistry. 2019; 44(4): 487–491, doi: 10.1515/tjb-2019-0057.
15. Zhao X, Wang Y, Sun X. The functions of microRNA-208 in the heart. Diabetes Res Clin Pract. 2020; 160: 108004, doi: 10.1016/j.diabres.2020.108004, indexed in Pubmed: 31911250.

16. Króliczewski J, Sobolewska A, Lejnowski D, et al. microRNA single polynucleotide polymorphism influences on microRNA biogenesis and mRNA target specificity. Gene. 2018; 640: 66–72, doi: 10.1016/j.gene.2017.10.021, indexed in Pubmed: 29032146.
17. Zhang B, Pan X, Cobb GP, et al. microRNAs as oncogenes and tumor suppressors. Dev Biol. 2007; 302(1): 1–12, doi: 10.1016/j.ydbio.2006.08.028, indexed in Pubmed: 16989803.
18. Marsh EE, Lin Z, Yin P, et al. Differential expression of microRNA species in human uterine leiomyoma versus normal myometrium. Fertil Steril. 2008; 89(6): 1771–1776, doi: 10.1016/j.fertnstert.2007.05.074, indexed in Pubmed: 17765232.
19. Hu H, Wang G, Li C. miR-124 suppresses proliferation and invasion of nasopharyngeal carcinoma cells through the Wnt/β-catenin signaling pathway by targeting Capn4. Onco Targets Ther. 2017; 10: 2711–2720, doi: 10.2147/OTT.S135563, indexed in Pubmed: 28579809.
20. Pençe H. Investigation of LFA-1 rs2230433 variation in renal cell carcinoma tissues. Haydarpasa Numune Training and Research Hospital Medical Journal. 2019; 59(3): 216–219, doi: 10.14744/hnhj.2019.63325.
21. Alsaadoni H, Caykara B, Pence S, et al. Analysis of the BACE1 and Clusterin Genes Expression Levels in Alzheimer's Disease. Journal of Academic Research in Medicine. 2019; 9(1): 45–49, doi: 10.5152/jarem.2019.2671.
22. Dogan H, Can H, Otu HH. Whole genome sequence of a Turkish individual. PLoS One. 2014; 9(1): e85233, doi: 10.1371/journal.pone.0085233, indexed in Pubmed: 24416366.
23. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001; 25(4): 402–408, doi: 10.1006/meth.2001.1262.
24. Di Tommaso S, Massari S, Malvasi A, et al. Selective genetic analysis of myoma pseudocapsule and potential biological impact on uterine fibroid medical therapy. Expert Opin Ther Targets. 2015; 19(1): 7–12, doi: 10.1517/14728222.2014.975793, indexed in Pubmed: 25363374.
25. Di Tommaso S, Massari S, Malvasi A, et al. Gene expression analysis reveals an angiogenic profile in uterine leiomyoma pseudocapsule. Mol Hum Reprod. 2013; 19(6): 380–387, doi: 10.1093/molehr/gat007, indexed in Pubmed: 23355533.
26. Serrat N, Sebastian C, Pereira-Lopes S, et al. The response of secondary genes to lipopolysaccharides in macrophages depends on histone deacetylase and phosphorylation of C/EBPβ. J Immunol. 2014; 192(1): 418–426, doi: 10.4049/jimmunol.1203500, indexed in Pubmed: 24307736.
27. Tanoğlu EG, Pençe H, Karuserci ÖK, et al. Uterin leiomyomda Wnt, β-katenin, TGF–β, Siklin D1'in ekspresyon seviyelerinin belirlenmesi. Zeynep Kamil Tıp Bülteni. 2019; 50(3): 138–141, doi: 10.16948/zktipb.629373.
28. Chegini N. Uterine microRNA Signature and Consequence of Their Dysregulation in Uterine Disorders. Anim Reprod. 2010; 7(3): 117–128.
29. Park MJu, Shen H, Spaeth JM, et al. Oncogenic exon 2 mutations in Mediator subunit MED12 disrupt allosteric activation of cyclin C-CDK8/19. J Biol Chem. 2018; 293(13): 4870–4882, doi: 10.1074/jbc.RA118.001725, indexed in Pubmed: 29440396.
30. Graaff Mde, Cleton-Jansen AM, Szuhai K, et al. Mediator complex subunit 12 exon 2 mutation analysis in different subtypes of smooth muscle tumors confirms genetic heterogeneity. Human Pathology. 2013; 44(8): 1597–1604, doi: 10.1016/j.humpath.2013.01.006.
31. Pérot G, Croce S, Ribeiro A, et al. MED12 alterations in both human benign and malignant uterine soft tissue tumors. PLoS One. 2012; 7(6): e40015, doi: 10.1371/journal.pone.0040015, indexed in Pubmed: 22768200.
32. Zhang B, Pan X, Cobb GP, et al. microRNAs as oncogenes and tumor suppressors. Dev Biol. 2007; 302(1): 1–12, doi: 10.1016/j.ydbio.2006.08.028, indexed in Pubmed: 16989803.
33. Subramanian S, Lui WO, Lee CH, et al. MicroRNA expression signature of human sarcomas. Oncogene. 2008; 27(14): 2015–2026, doi: 10.1038/sj.onc.1210836, indexed in Pubmed: 17922033.
34. Marsh EE, Lin Z, Yin P, et al. Differential expression of microRNA species in human uterine leiomyoma versus normal myometrium. Fertil Steril. 2008; 89(6): 1771–1776, doi: 10.1016/j.fertnstert.2007.05.074, indexed in Pubmed: 17765232.
35. Wang T, Zhang X, Obijuru L, et al. A micro-RNA signature associated with race, tumor size, and target gene activity in human uterine leiomyomas. Genes Chromosomes Cancer. 2007; 46(4): 336–347, doi: 10.1002/gcc.20415, indexed in Pubmed: 17243163.
36. Zhang G, Song K, Yan H. MicroRNA-124 represses wound healing by targeting SERP1 and inhibiting the Wnt/β-catenin pathway. Adv Clin Exp Med. 2019; 28(6): 711–718, doi: 10.17219/acem/94163, indexed in Pubmed: 30740945.
37. Zhang X, Cai D, Meng L, et al. MicroRNA-124 inhibits proliferation, invasion, migration and epithelial-mesenchymal transition of cervical carcinoma cells by targeting astrocyte-elevated gene-1. Oncol Rep. 2016; 36(4): 2321–2328, doi: 10.3892/or.2016.5025, indexed in Pubmed: 27571703.
38. Yuan Li, Li S, Zhou Qi, et al. MiR-124 inhibits invasion and induces apoptosis of ovarian cancer cells by targeting programmed cell death 6. Oncol Lett. 2017; 14(6): 7311–7317, doi: 10.3892/ol.2017.7157, indexed in Pubmed: 29344168.
39. Lu ML, Zhang y, Li J, et al. MicroRNA-124 Inhibits Colorectal Cancer Cell Proliferation and Suppresses Tumor Growth by Interacting With PLCB1 and Regulating Wnt/β-catenin Signaling Pathway. Eur Rev Med Pharmacol Sci. 2019; 23(1): 121–136.
40. Du S, Li H, Sun X, et al. MicroRNA-124 inhibits cell proliferation and migration by regulating SNAI2 in breast cancer. Oncol Rep. 2016; 36(6): 3259–3266, doi: 10.3892/or.2016.5163, indexed in Pubmed: 27748910.
41. Yu Bo, Jiang K, Zhang J. MicroRNA-124 suppresses growth and aggressiveness of osteosarcoma and inhibits TGF-β-mediated AKT/GSK-3β/SNAIL-1 signaling. Mol Med Rep. 2018; 17(5): 6736–6744, doi: 10.3892/mmr.2018.8637, indexed in Pubmed: 29488603.
42. Cui Z, Hu Y. MicroRNA-124 Suppresses Slug-mediated Lung Cancer Metastasis. Eur Rev Med Pharmacol Sci. 2016; 20(18): 3802–3811.
43. Xiao HJ, Ji Q, Yang L, et al. In vivo and in vitro effects of microRNA-124 on human gastric cancer by targeting JAG1 through the Notch signaling pathway. J Cell Biochem. 2018; 119(3): 2520–2534, doi: 10.1002/jcb.26413, indexed in Pubmed: 28941308.
44. Wilting SM, van Boerdonk RAA, Henken FE, et al. Methylation-mediated silencing and tumour suppressive function of hsa-miR-124 in cervical cancer. Mol Cancer. 2010; 9: 167, doi: 10.1186/1476-4598-9-167, indexed in Pubmed: 20579385.
45. Banno K, Yanokura M, Iida M, et al. Carcinogenic mechanisms of endometrial cancer: involvement of genetics and epigenetics. J Obstet Gynaecol Res. 2014; 40(8): 1957–1967, doi: 10.1111/jog.12442, indexed in Pubmed: 25131761.
46. Chegini N. Proinflammatory and Profibrotic Mediators: Principal Effectors of Leiomyoma Development as a Fibrotic Disorder. Seminars in Reproductive Medicine. 2010; 28(04): 345–346, doi: 10.1055/s-0030-1255183.
47. Nair S, Al-Hendy A. Adipocytes enhance the proliferation of human leiomyoma cells via TNF-α proinflammatory cytokine. Reprod Sci. 2011; 18(12): 1186–1192, doi: 10.1177/1933719111408111, indexed in Pubmed: 22096007.
48. Wang T, Zhang X, Obijuru L, et al. A micro-RNA signature associated with race, tumor size, and target gene activity in human uterine leiomyomas. Genes Chromosomes Cancer. 2007; 46(4): 336–347, doi: 10.1002/gcc.20415, indexed in Pubmed: 17243163.
49. Zhang G, Song K, Yan H. MicroRNA-124 represses wound healing by targeting SERP1 and inhibiting the Wnt/β-catenin pathway. Adv Clin Exp Med. 2019; 28(6): 711–718, doi: 10.17219/acem/94163, indexed in Pubmed: 30740945.
50. Santamaria X, Taylor H. Micro RNA and gynecologicalr eproductive diseases. Fertility and Sterility. 2014; 101(6): 1545–1551.
51. Chegini N. Uterine microRNA signature and consequence of their dysregulation in uterine disorders. Anim Reprod. 2010; 7(3): 117–128, indexed in Pubmed: 22328907.
52. Sun Y, Li Qi, Gui H, et al. MicroRNA-124 mediates the cholinergic anti-inflammatory action through inhibiting the production of pro-inflammatory cytokines. Cell Res. 2013; 23(11): 1270–1283, doi: 10.1038/cr.2013.116, indexed in Pubmed: 23979021.
53. Ponomarev ED, Veremeyko T, Barteneva N, et al. MicroRNA-124 promotes microglia quiescence and suppresses EAE by deactivating macrophages via the C/EBP-α-PU.1 pathway. Nat Med. 2011; 17(1): 64–70, doi: 10.1038/nm.2266, indexed in Pubmed: 21131957.
54. Hu H, Wang G, Li C. miR-124 suppresses proliferation and invasion of nasopharyngeal carcinoma cells through the Wnt/β-catenin signaling pathway by targeting Capn4. Onco Targets Ther. 2017; 10: 2711–2720, doi: 10.2147/OTT.S135563, indexed in Pubmed: 28579809.
55. Zhang C, Hu Y, Wan J, et al. MicroRNA-124 suppresses the migration and invasion of osteosarcoma cells via targeting ROR2-mediated non-canonical Wnt signaling. Oncol Rep. 2015; 34(4): 2195–2201, doi: 10.3892/or.2015.4186, indexed in Pubmed: 26259653.
56. Long QZ, Du YF, Liu XG, et al. miR-124 represses FZD5 to attenuate P-glycoprotein-mediated chemo-resistance in renal cell carcinoma. Tumour Biol. 2015; 36(9): 7017–7026, doi: 10.1007/s13277-015-3369-3, indexed in Pubmed: 25861751.