Original Article
http://mjiri.iums.ac.ir

Medical Journal of the Islamic Republic of Iran (MJIRI)
Iran University of Medical Sciences

Does *PTEN* gene mutation play any role in Li-Fraumeni syndrome?

Mansoureh Akouchekian*[1], Simin Hemati[2], Davood Jafari[3], Nazanin Jalilian[4]
Masoumeh Dehghan Manshadi[5]

Received: 1 July 2015 Accepted: 18 November 2015 Published: 29 May 2016

Abstract

Background: Li-Fraumeni syndrome (LFS) is one of the most serious hereditary cancer syndromes with a high risk of malignancy in childhood. This syndrome is an autosomal dominant cancer predisposing syndrome due to a germline mutation in the *TP53* tumor suppressor gene.

Methods: In this study, a representative family case of Li-Fraumeni syndrome is described. The proband of this family was a 43-year-old male who had osteosarcoma of the mandible and a positive family history of cancer. His mother died at the age of 29 of brain cancer; his sister died at the age of 18 of breast cancer; his brother died at the age of 36 of liver cancer; and another sister of his died at the age of 16 of leukemia. Complete sequence analysis of the *TP53* and *PTEN* genes was performed in this family. We used standard diagnostic tools such as sequencing and multiplex ligation-dependent probe amplification (MLPA) to analyze these two genes in this family. The exons and flanking exon-intron junctions of the *TP53* and *PTEN* genes were sequenced.

Results: We detected a germline mutation in the *TP53* gene in this family that was previously reported as somatic mutation in LFS in the catalogue of somatic mutations in cancer (COSMIC). In addition, according to the International Agency for Research of Cancer (IARC) database, a 19-year-old male patient with sarcoma was recently reported to have this germline mutation. We also found two new IVS variations in the *PTEN* gene, one of which can be a suggestive evidence of an effect on the splicing of PTEN.

Conclusion: Genomic modifications for tumor risk and genotype-phenotype correlations in LFS are still to be identified. We believe every new finding in this area can provide new insights into the pathogenesis and progression of Li-Fraumeni syndrome.

Keywords: *PTEN* Gene, Li-Fraumeni Syndrome, Germline Mutation.

Cite this article as: Akouchekian M, Hemati S, Jafari D, Jalilian N, Dehghan Manshadi M. Does *PTEN* gene mutation play any role in Li-Fraumeni syndrome?. *Med J Islam Repub Iran* 2016 (29 May). Vol. 30:378.

Introduction

Li-Fraumeni syndrome (LFS) was first described by Li and Fraumeni in 1969 (1). LFS is a rare, familial, autosomal-dominant disease and is characterized by the development of breast cancer, leukemia, sarcoma, and other neoplasms in children and young adults (1,2).

LFS syndrome in its classic form is characterized by a proband with sarcoma before the age of 45, a first-degree relative with any cancer before the age of 45, another first or second degree relative with any cancer before the age of 45, or with sarcoma at any age (3). A 1994 Birsh et al. publication described families who were predisposed to LFS, but did not precisely meet the classic diagnostic criteria. They proposed a Li-Fraumeni-like syndrome based on a more detailed classification of the age at onset and tumor type (4). In the 1990s, through the genetic analyses of

[1]. (**Corresponding author**) PhD, Assistant Professor, Department of Medical Genetics & Molecular Biology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran. akouchekian.m@iums.ac.ir
[2]. MD, PhD, Associate Professor, Department of Oncology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran. hematti@med.mui.ac.ir
[3]. MSc, PhD student, Department of Immunology, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran. davoodjafari1@yahoo.com
[4]. PhD, Department of Medical Genetics, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran. jalilian_n@razi.tums.ac.ir
[5]. MSc, Medical Genetics Department, Special Medical Center, Tehran, Iran. m_dehghanmanshadi@yahoo.com

many Li-Fraumeni syndrome (LFS) families, it was revealed that about 70% of patients had germline mutations in the *TP53* tumor suppressor gene (5,6).

The most frequent soft tissue sarcomas in LFS are rhabdomyosarcomas, leiomyosarcomas, liposarcomas, fibrous histiocytomas, and fibrosarcomas (7). The sarcomas tend to occur most frequently in childhood, but individuals with LFS are still at risk for developing sarcomas in adulthood. LFS patients are at higher risk for osteosarcomas as well. Leukemia is seen with increased frequency in LFS, and some of its types are acute lymphocytic leukemia, acute myelocytic leukemia, and chronic myelocytic leukemia (7).

Germline mutations of the *TP53* tumor suppressor gene is a cause of LFS, and the finding of such a mutation can be useful as a marker of increased susceptibility to the tumor spectrum of the syndrome (7-9). Another gene may account for families without detectable germline *TP53* mutations. The *TP53* tumor suppressor gene has multiple functions, among which are controlling cell cycle progression and regulation of the cellular response to DNA damage (10). Commercial genetic tests are based on sequence analysis in the exon 5-9, in which 95% of mutations occur.

Depending on the mutation, different elements of normal *TP53*-mediated responses can be lost and some mutants can gain new non-wild-type functions. *TP53* is primarily known for its crucial role in the stress response of the cell to multiple insults, and it is a key regulator of cell cycle arrest, apoptosis, senescence, and DNA repair (11,12). The pleiotropic roles of *TP53* are still being elucidated, and in a recent work it was found that *TP53* has a role in ageing, immune response, and cell metabolism (3,13). *PTEN,* as a tumor suppressor gene, encodes a dual-specificity phosphatase with lipid phosphatase and protein tyrosine phosphatase activities that regulate cell growth and apoptosis as well as various other functions associated with carcinogenesis such as cell signaling, cell migration, and cellular adhesion to matrix (14-16). It is located on 10q23.3 and has nine exons. The protein encoded in this gene is a phosphatidylinositol l-3, 4, 5-trisphosphate 3-phosphatase. It contains a tensin-like domain as well as a catalytic domain similar to that of the dual-specificity protein- tyrosine phosphatases. Unlike most of the protein tyrosine phosphatases, this protein preferentially dephosphorylates phosphoinositide substrates. It negatively regulates intracellular levels of phosphatidylinositol-3, 4, 5-trisphosphate in cells and functions as a tumor suppressor by negatively regulating the AKT/PKB signaling pathway. In addition, it seems to have two different roles in cytoplasm and nucleus of the cell: Keeping the basal levels of PIP3 below a threshold for the PI3K/AKT signaling pathway activation in cytoplasm, and localization to the nucleus to bind and regulate p53 protein level and perform a transcriptional activity. Oxidative stress can be physiological stimuli that regulate the accumulation of nuclear *PTEN*. Nuclear *PTEN*, independent of its phosphatase activity, leads to p53-mediated G1 growth arrest, cell death, and reduction of reactive oxygen species production (17). The *PTEN* gene is an important tumor suppressor gene that shows both germline and somatic mutations in a variety of human tumor types (18, 19). The literature on the role of PTEN on LFS is controversial. A 1999 Burt et al. publication excluded PTEN as a candidate for mutation in LFS (20).

Tumor suppressor gene *TP53* is the most commonly mutated gene in human cancers and one of the most thoroughly studied (14, 21, 22). It is located on 17p13 and has 11 exons, and its monomer is a 393-amino acid protein with five domains. They are as follows: An N-terminal transactivation domain (amino acids 1–42); a proline-rich domain (amino acids 61–92); a central site-specific DNA-binding domain (amino acids 101–300); a tetramerization domain (tetramerization domain, amino acids 326–356); and a C-terminal basic domain (amino acids 364–393). Several stressors, including DNA damage, activate *TP53* partly

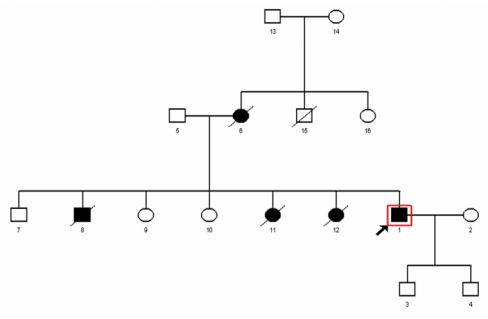

Fig. 1. Pedigree of the Family Carrying the D281E Germline Mutation in the *TP53* Gene and Two Substitutions in the *PTEN* Gene (IVS1-1G>A and IVS2+65 G>A)

through multiple post-translational modifications modulating its activity and stability (23).

In this study, we aimed to describe a representative family case of classic Li-Fraumeni syndrome. The proband of this family was a 43-year-old male who had osteosarcoma of the mandible and a positive family history of cancer. His mother died at the age of 29 of brain cancer; his sister died at the age of 18 of breast cancer; his brother died at the age of 36 of liver cancer; and another sister of his died at the age of 16 of leukemia. The proband's uncle passed away due to an old age and did not have any particular disease, but his aunt is still alive and is around 60 years old and does not have any disease (See family tree in Fig. 1).

Methods

Patients

Studies were conducted on the patient and his deceased 36-year-old brother and one of his sisters who was alive. An informed consent was obtained according to Iran University's ethical committee codes. The sample of other family members shown in the pedigree in Fig. 1 was not available. After detailed analysis of the family history and medical records of the affected individuals, we used standard diagnostic tools such as sequencing and multiplex ligation-dependent probe amplification (MLPA) to analyze the *TP53* gene in this family.

PCR Amplification of TP53 and PTEN Genes

Genomic DNA was extracted, and promoter regions, 11 exons of the *TP53* gene, and nine exons of the *PTEN* gene were amplified using independent PCR runs. PCR amplification was carried out in a final volume of 25µl containing 200-300 ng total DNA and 12.5ul CinaGen PCR Master Kit Cat. No. PR8251C (CinaGen, Tehran, Iran) and 10 pmol of each primer (Table 1). After initial denaturation for 5 min at 95°C, 38 cycles of amplification were performed as follows: 55s at 95°C, 50s at 52°C - 60°C and 55s at 72°C followed by 72°C for 10 min. PCR products were evaluated on 1.5% agarose gel followed by EtBr staining.

DNA Sequencing

Sequence analysis of PCR products from promoter region and all exons were done after purification of PCR products (PCR product purification kit, Roche). Both strands were sequenced by Big Dye Termination system in a directly determined automated sequencing on an ABI 3700 capillary sequencer machine using both primers (Macrogene, Seoul, Korea). Sequencing results were analyzed using bioinformatics' tools, Sequencher Software 5.

MLPA

Multiplex ligation-dependent probe amplification (MLPA) is used to identify large deletions and duplications that are not detectable by sequence analysis. MLPA is routinely performed for *TP53* using commercially available kits (MRC-Holland, Amsterdam and the Netherlands) (24). Briefly, a probe mix of oligonucleotide pairs, with each pair directed to a specific target (e.g., an exon of a gene is hybridized to genomic DNA), allows the ligation of adjacent probes. Subsequently, ligation products can be amplified by PCR using universal sequence tags and can be discriminated by size due to included stuffer sequences. The amount of PCR products in comparison to control samples allows the identification of deletions or duplications of

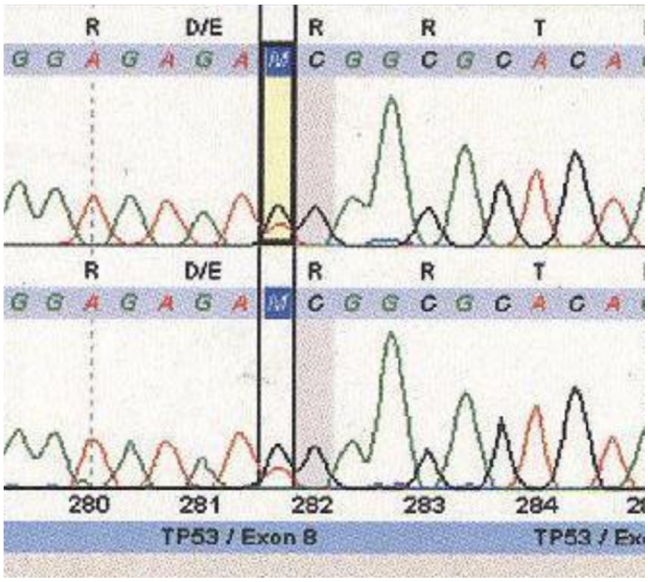

Fig. 2. Exon 8 Mutation in the *TP53* Gene, C>A Substitution→ D281E

target sites; e.g., whole exons (8,25-26).

Results

We completed whole gene sequencing for the *TP53* and *PTEN* genes in this family. We detected a germline mutation in the *TP53* gene in this family that was previously reported as somatic mutation in LFS in the IARC database (Fig. 2). In the *PTEN* gene, we found two germline sequence variants as single-nucleotide substitutions, one in the splice site acceptor of intron 1 of the *PTEN* gene (IVS1-1G>A) (Fig. 3) and the other in IVS2+65 G>A (Fig. 4). The paraffin-embedded liver cancer sample of the brother who passed away at the age of 36 from liver cancer was studied in both genes, and mutations existed in them. We analyzed both the new changes found in the *PTEN* gene in the two sites of NetGene 2 and Alternative Splice Site Predictor (ASSP). When the IVS1-1G>A change is imposed in NetGene2 site, the 3′ splice site acceptor (SSA) is removed from the consensus sequences, meaning that the IVS1-1G>A variation could cause a splicing site mutation, but the IVS 2+65 G>A variation does not cause a new change in splicing site.

Table 1. Primer Sequences of TP53 and PTEN

Gene	Exon	Primer sequence 5′→3′	Tm (°C)	Product size (bp)
PTEN	1(Forward)	CAAGTCCAGAGCCATTTCCATC	55	297
	1(Reverse)	GCAACCTGACCAGGGTTAAATG	55	297
	2(Forward)	CTCCAGCTATAGTGGGGAAAAC	55	361
	2(Reverse)	GTCCATTAGGTACGGTAAGCCA′	55	361
	3(Forward)	CTACTCTAAACCCATAGAAGGG	53	308
	3(Reverse)	CTTGGACTTCTTGACTTAATCGG	53	308
	4(Forward)	GGGGGTGATAACAGTATCTACT′	53	285
	4(Reverse)	CAGTAAGATACAGTCTATCGGG′	53	285
	5(Forward)	CTCTGGAATCCAGTGTTTCTTT	52	422
	5(Reverse)	CCAATAAATTCTCAGATCCAGG′	52	422
	6(Forward)	CTACGACCCAGTTACCATAGCA	55	415
	6(Reverse)	GGCTTCTTTAGCCCAATGAGTTG	55	415
	7(Forward)	GCTTGAGATCAAGATTGCAG	50	439
	7(Reverse)	CAATGCCAGAGTAAGCAAAAC	50	439
	8(Forward)	CAACAGATAACTCAGATTGCC	53	506
	8(Reverse)	GTTCTTCATCAGCTGTACTCCT	53	506
	9(Forward)	GAGGGTCATTTAAAAGGCCTCT	53	458
	9(Reverse)	CTGGTAATCTGACACAATGTCC	53	458

Table 1. Cntd

TP53	2-3(Forward)	TCTCATGCTGGATCCCCACT	58	344
	2-3(Reverse)	AGTCAGAGGACCAGGTCCTC	58	344
	4(Forward)	TGAGGACCTGGTCCTCTGAC	57	413
	4 (Reverse)	AGAGGAATCCCAAAGTTCCA	57	413
	5-6 (Forward)	TGTTCACTTGTGCCCTGACT	59	467
	5-6 (Reverse)	TTAACCCCTCCTCCCAGAGA	59	467
	7(Forward)	CTTGCCACAGGTCTCCCCAA	60	237
	7(Reverse)	AGGGGTCAGAGGCAAGCAGA	60	237
	8-9(Forward)	TTGGGAGTAGATGGAGCCT	59	455
	8-9(Reverse)	AGTGTTAGACTGGAAACTT	59	455
	10 (Forward)	CAATTGTAACTTGAACCATC	55	260
	10(Reverse)	GGATGAGAATGGAATCCTAT	55	260
	11(Forward)	AGACCCTCTCACTCATGTGA	59	245
	11(Reverse)	TGACGCACACCTATTGCAAG	59	245

Discussion

The *PTEN*, *TP53* genes play an important role in the development of cancers. However, the role of genetic variations of this tumor suppressor-oncoprotein network in LFS is not yet fully understood. *TP53* and *PTEN* are the most commonly altered tumor suppressor genes in human cancers; however, the mutations spectrum of this two tumor suppressor genes are distinguished. The mutation of *TP53* gene occurs frequently in colon, lung and breast cancers, while mutations in the PTEN gene are found in prostate cancer, malignant melanoma and glioblastoma. *TP53* mutation, as a germline mutation, is reported in a familial syndrome of breast cancer, sarcoma and other neoplasms (27, 28). The aim of this study was to genetically characterize *TP53* and *PTEN* tumor suppressor genes in LFS.

Nuclear *PTEN* has previously been demonstrated to control chromosome stability and DNA repair (29). Previous studies showed a direct binding of *TP53* to a site on the *PTEN* promoter, suggesting that *TP53* regulates *PTEN* by transcription (30).

Loss of *PTEN* expression has been previously correlated both with a favorable and unfavorable prognosis, and inactivation of this gene has been found not only in early-stage well-differentiated carcinomas, but also in advanced and invasive endometrial

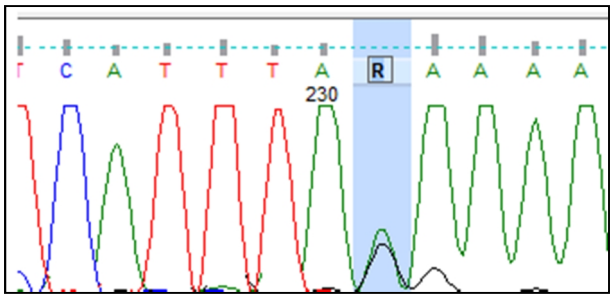

Fig. 3. Heterozygous IVS2+65 G>A of the *PTEN* Gene

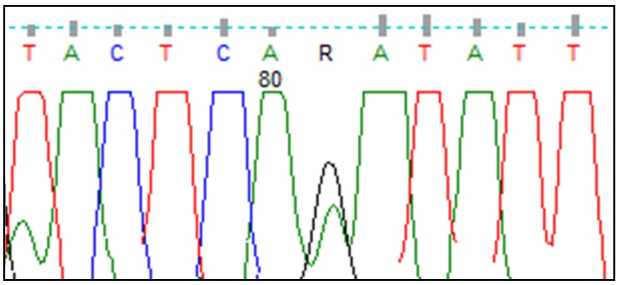

Fig. 4. Heterozygous IVS1-1G>A change of the 3′ Splice Site Acceptor (SSA) of the *PTEN* Gene

tumors (31).

Among the *PTEN* polymorphisms identified to date, one is in 5′UTR region (–9C/G), and two are in introns (IVS4 and IVS7). The substitution of C to G in the 5′UTR results in a recovery of the periodical occurrence of the G residue (gtcccagacATGa), which closely matches the consensus sequence that helps ribosomes stay in the frame during translation, and it may affect the expression of the *PTEN* gene (32). The polymorphism of the 5 bp (ATCTT) insertion is the downstream of exon 4 in intron 4. Although the function of this polymorphism is still unknown, the variant position may lead to a splicing error or may affect the function of the *PTEN* through linkage disequilibrium with another variant (32).

The interaction and cooperation between the *TP53* and *PTEN* genes in their respective pathways are necessary because both of them are essential guardians of the human genome. These two tumor suppressor genes act differently when guarding the human genome. Expression of PTEN is high in cells and tissues, and it acts like a police force, but *TP53* are usually extremely low but are highly increased following DNA damage and genotoxic stress (33).

The inheritance pattern in the family suggests that it is indeed a monogenic disorder, autosomal dominant inherited, and the D281E mutation in *TP53* gene is the top candidate mutation to be the cause of LFS in this family. *TP53* is located on chromosome 17 while *PTEN* is located on chromosome 10. Therefore, the variation of these two genes is not linked, and we could assume that except for the *TP53* D281E, not all the affected individuals of the family had the *PTEN* variants. However, the samples of other affected family members, except for the deceased 36-year-old brother, were not available to confirm this hypothesis. Accordingly, the non-affected sister of the family did not have the *TP53* or the *PTEN* mutations.

The presumptive splice alteration by IVS1-1G>A could be tested by RT-PCR. For RT-PCR, fresh blood was needed to extract RNA, but the patient was living in another city about 500 kilometers far from Tehran and refused to come to Tehran for sampling. Unfortunately, despite much efforts and spending a lot of time, we could not make new sampling to test the possible splice alteration by IVS1-1G>A. Therefore, we can propose that the mentioned variation might have an effect on the splicing of PTEN.

Recently, scientists have found that genetic variations within the *PTEN*, *AKT1*, *MDM2,* and *TP53* networks can be used as biomarkers to identify high-risk subgroups of patients who might benefit from personalized prevention and treatment (34). They also concluded that numerous interactions might support the biological plausibility that the combination of variants of the *PTEN*, *AKT1*, *MDM2,* and *TP53* networks could result in more comprehensive and accurate estimates of the risk for carcinoma than can be obtained from a single variant(34).

Conclusion

Based on our findings in this study, we can argue that the genetic variants of the *PTEN* and *TP53* genes may jointly influence more susceptibility to LFS risk or may exacerbate the symptoms in patients who have germline variations in these two genes.

Acknowledgements

We are deeply grateful to the Institute of Cell and Molecular Pathology at Hannover Medical School in Germany where the experimental analysis of the *PT53* gene of the proband of the family was done. We also thank the family who allowed us to do this study.

References

1. Li FP, Fraumeni Jr JF. Soft-tissue sarcomas, breast cancer, and other neoplasms. A familial syndrome? Annals of internal medicine 1969; 71(4):747-52.

2. Li FP, Fraumeni Jr JF. Prospective study of a

family cancer syndrome. JAMA : the journal of the American Medical Association 1982;247(19):2692-4.

3. Li FP, Fraumeni Jr JF, Mulvihill JJ, Blattner WA, Dreyfus MG, Tucker MA, et al. A cancer family syndrome in twenty-four kindreds. Cancer research 1988;48(18):5358-62.

4. Birch JM, Hartley AL, Tricker KJ, Prosser J, Condie A, Kelsey AM, et al. Prevalence and diversity of constitutional mutations in the p53 gene among 21 Li-Fraumeni families. Cancer research 1994;54(5):1298-304.

5. Brown LT, Sexsmith E, Malkin D. Identification of a novel PTEN intronic deletion in Li-Fraumeni syndrome and its effect on RNA processing. Cancer genetics and cytogenetics 2000; 123(1):65-8.

6. Varley JM. Germline TP53 mutations and Li-Fraumeni syndrome. Human mutation 2003; 21(3):313-20.

7. Carrie P. Hunter, Karen A Johnson, Hyman B. Muss. Cancer in the Elderly 2005.

8. Naguib A, Bencze G, Engle DD, Chio, II, Herzka T, Watrud K, et al. p53 mutations change phosphatidylinositol acyl chain composition. Cell reports 2015;10(1):8-19.

9. Gonzalez-Billalabeitia E, Seitzer N, Song SJ, Song MS, Patnaik A, Liu XS, et al. Vulnerabilities of PTEN-p53-deficient prostate cancers to compound PARP/PI3K inhibition. Cancer discovery 2014.

10. Levine AJ. p53, the cellular gatekeeper for growth and division. Cell 1997;88(3):323-31.

11. Memmel S, Sukhorukov VL, Horing M, Westerling K, Fiedler V, Katzer A, et al. Cell surface area and membrane folding in glioblastoma cell lines differing in PTEN and p53 status. PloS one 2014;9(1):e87052.

12. Birch JM, Hartley AL, Blair V, Kelsey AM, Harris M, Teare MD, et al. Cancer in the families of children with soft tissue sarcoma. Cancer 1990; 66(10):2239-48.

13. Malkin D. Li-fraumeni syndrome. Genes & cancer. 2011;2(4):475-84.

14. Li X, Xie W, Xie C, Huang C, Zhu J, Liang Z, et al. Curcumin Modulates miR-19/PTEN/AKT/p53 Axis to Suppress Bisphenol A-induced MCF-7 Breast Cancer Cell Proliferation. Phytotherapy research: PTR 2014.

15. Heinze B, Herrmann LJ, Fassnacht M, Ronchi CL, Willenberg HS, Quinkler M, et al. Less common genotype variants of TP53 polymorphisms are associated with poor outcome in adult patients with adrenocortical carcinoma. European journal of endocrinology / European Federation of Endocrine Societies 2014;170(5):707-17.

16. Yamada KM, Araki M. Tumor suppressor PTEN: modulator of cell signaling, growth, migration and apoptosis. Journal of cell science 2001;114(Pt 13):2375-82.

17. Chang CJ, Mulholland DJ, Valamehr B, Mosessian S, Sellers WR, Wu H. PTEN nuclear localization is regulated by oxidative stress and mediates p53-dependent tumor suppression. Molecular and cellular biology 2008;28(10):3281-9.

18. Leslie NR, Downes CP. PTEN: The down side of PI 3-kinase signalling. Cellular signalling 2002;14(4):285-95.

19. Waite KA, Eng C. Protean PTEN: form and function. American journal of human genetics 2002;70(4):829-44.

20. Burt EC, McGown G, Thorncroft M, James LA, Birch JM, Varley JM. Exclusion of the genes CDKN2 and PTEN as causative gene defects in Li-Fraumeni syndrome. British journal of cancer 1999;80(1-2):9-10.

21. Kim G, Ouzounova M, Quraishi AA, Davis A, Tawakkol N, Clouthier SG, et al. SOCS3-mediated regulation of inflammatory cytokines in PTEN and p53 inactivated triple negative breast cancer model. Oncogene 2014.

22. Mazurek A, Pierzynski P, Kuc P, Kopinski P, Terlikowski S, Niklinska W, et al. Evaluation of angiogenesis, p-53 tissue protein expression and serum VEGF in patients with endometrial cancer. Neoplasma 2004;51(3):193-7.

23. Meek DW, Anderson CW. Posttranslational modification of p53: cooperative integrators of function. Cold Spring Harbor perspectives in biology 2009;1(6):a000950.

24. Schouten JP, McElgunn CJ, Waaijer R, Zwijnenburg D, Diepvens F, Pals G. Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. Nucleic acids research 2002;30(12):e57.

25. Ou WB, Zhu J, Eilers G, Li X, Kuang Y, Liu L, et al. HDACi inhibits liposarcoma via targeting of the MDM2-p53 signaling axis and PTEN, irrespective of p53 mutational status. Oncotarget 2015;6(12):10510-20.

26. Ripperger T, Troger HD, Schmidtke J. The genetic message of a sudden, unexpected death due to thoracic aortic dissection. Forensic science international 2009;187(1-3):1-5.

27. Yin Y, Shen WH. PTEN: a new guardian of the genome. Oncogene 2008;27(41):5443-53.

28. Malkin D, Li FP, Strong LC, Fraumeni JF, Jr., Nelson CE, Kim DH, et al. Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. Science (New York, NY) 1990;250(4985):1233-8.

29. Shen WH, Balajee AS, Wang J, Wu H, Eng C, Pandolfi PP, et al. Essential role for nuclear PTEN in maintaining chromosomal integrity. Cell 2007; 128(1):157-70.

30. Stambolic V, MacPherson D, Sas D, Lin Y, Snow B, Jang Y, et al. Regulation of PTEN transcription by p53. Molecular cell 2001;8(2):317-25.

31. Ali IU, Schriml LM, Dean M. Mutational

spectra of PTEN/MMAC1 gene: a tumor suppressor with lipid phosphatase activity. Journal of the National Cancer Institute 1999;91(22):1922-32.

32. Ge H, Cao YY, Chen LQ, Wang YM, Chen ZF, Wen DG, et al. PTEN polymorphisms and the risk of esophageal carcinoma and gastric cardiac carcinoma in a high incidence region of China. Diseases of the esophagus : official journal of the International Society for Diseases of the Esophagus/ ISDE 2008;21(5):409-15.

33. Yin L, Liu CX, Nong WX, Chen YZ, Qi Y, Li HA, et al. Mutational analysis of p53 and PTEN in soft tissue sarcoma. Molecular medicine reports 2012;5(2):457-61.

34. Zhang X, Chen X, Zhai Y, Cui Y, Cao P, Zhang H, et al. Combined effects of genetic variants of the PTEN, AKT1, MDM2 and p53 genes on the risk of nasopharyngeal carcinoma. PloS one 2014; 9(3):e92135.