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Research article

STUDY ON RELATIONSHIP BETWEEN OVINE PULMONARY ADENOCARCINOMA AND P53 TUMOR SUPPRESSOR GENE MUTATION

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ABSTRACT: P53 also known as tumor suppression protein has three main important roles such as growth arrest, apoptosis and DNA repair. Damaged p53 in cells by mutagens (radiation, chemicals or viruses) resulting in cancer. Ovine pulmonary adenomatosis (OPA) also known Jaagsiekte, is a contagious tumour of sheep that caused by a beta retrovirus (jaagsiekte sheep retrovirus: JSRV). The present study was described mutation of p53 gene (enter 7 and 8 exons) on the OPA pathological samples. Fifty pathological samples were collected from Tabriz-Iran slaughterhouse. Eleven out of fifty samples were diagnosed as OPA-affected according to necropsy finding by histopathology and immunohistochemistry. Then, total DNA was extracted from tissues and polymerase chain reaction was run by two pair specific primers. Amplifications products were screened using Sanger sequencing to finding point mutation. The results of this study were showed that there was no mutation through 7 & 8 exons of p53 tumor suppressor gene in ovine pulmonary adenomatosis disease. This is the first study of p53 gene (exons 7 & 8) on pathological samples of Iranian sheep (Gezel). More studies are needed to finding relationship between p53 and ovine pulmonary adenomatosis disease.

Key words: Mutation, P53 tumor suppressor gene, exons 7 & 8, Ovine pulmonary adenocarcinoma

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INTRODUCTION

Tumor suppressor produced a nuclear phosphor protein named P53 which is located on chromosome 11insheep and chromosome 17p13 on human (Kern et al., 1991; Matlashewski et al., 1984). P53 protein appeared in 53-kilodalton (kDa) molecular weight on SDS-PAGE analysis. But, its amino acid residues are only 43.7 kDa. For this reason, P53 protein containing high number of proline residues and proline migrate slowly on SDS-PAGE therefore its molecular weight appear heavier than it actually (Ziemer et al., 1982).

When P53 protein binds to DNA, it turn stimulates of translation p21 gene which produce a protein that interacts with cdk2 (cell division-stimulating protein), complexions of p21with cdk2 inhibit of passing the cell to next stage of cell division. Mutation within p53 result its inability of longer binding to DNA. Consequently the p21 protein is not made available to act as the 'stop signal' for cell division (National Center for Biotechnology Information., 2008)

P53 has managed many mechanisms such as anticancer function, genomic stability, apoptosis, and inhibition of angiogenesis (Ferreira et al., 1999).Mutations affected on ability of the protein to binding to target DNA sequences consequently resulting transcriptional activation prevention of mentioned genes (Brambilla& Brambilla., 1997).

Ovine pulmonary adenocarcinoma (OPA), as well known jaagsiekte (driving sickness), pulmonary adenomatosis or epizootic adenomatosis is a transmissible lung tumor which caused by jaagsiekte sheep retrovirus (JSRV) (Griffiths et al., 2010; Sharp et al., 2003).Sheep are susceptible at all ages; however clinical signs are commonly seen in mature sheep between 2 to 4 years old due to long incubation period(Griffiths et al., 2010; Tustin et al., 1996).Currently, there is no vaccination or treatment for clinical ovine pulmonary adenomatosis which is inevitably fatal (Mostafa et al., 2013).

In the lung, the tumor occurson secretory epithelial cells and be able to block lung function (Rezazadeh et al., 2012).Mutations often occur in specific region of p53 tumor suppressor gene in some cancers. Generally, mutation of the P53gene is one of the key prognostic factors in cell proliferation, DNA reparation and aging (Matlashewski et al., 1984). The p53 gene is a tumor suppressor gene; its activity is against uncontrollable cell growth resulting formation of tumors (Bates et al., 1998).

The TP53 gene can be damaged in cells by mutagens (chemicals, radiation, or viruses), increasing the likelihood that the cell will begin decontrolled division (Hollstein et al., 1991).The p53gene is the most frequently mutated gene in human and animal neoplasia with over 10 000 reported mutations (Gruszka-Westwood et al., 2001).

In veterinary most studies were performed on dogs, including thyroid carcinoma (Devilee et al., 1994). Osteosarcoma (Johnson et al., 1998) and breast tumors(Lee et al., 2004; Muto et al., 2000; Wakui et al., 2000) lymphoma (Veldhoen et al., 1998) that in all of these studies, mutations have been identified in tp53. Also, mutations of p53 have been identified horses' Squamous cell carcinoma (Teifke et al., 1996).In cows with BLV, PG53 mutations have been observed (Dequiedt et al., 1995; Ishiguro et al., 1997). In tumors of bovine digestive system changes of p53 genes have also been reported (Scobie et al., 1997).Therefore, it was needed to more studies on sheep'sp53 gene by considering within exon 7 and 8 to finding accession of these exons andovine pulmonary adenocarcinoma diseases. Thus, the aim of this study was to investigate the relationship between P53 gene mutations in exons 7, 8 with Ovine pulmonary adenocarcinoma in Gezel breed, by PCR and sequencing of PCR products. In this study, the pfu enzyme was used instead of taq polymerase that itsensitivities too high.

MATERIALS AND METHODS

In this study, a total numbers of50samples were collected from Gezel sheep (Iranians breed)in Tabriz-Iran slaughterhouse which diagnoses as tumor lung diseases by macroscopic inspection. Briefly**,** tissue samples from tumors lesions were removed after postmortem autopsy, immediately frozen in liquid nitrogen, and stored at -70^{oC}.

In the pathology laboratory, all tissue samples of recorded lung lesions were considered for pathological diagnostic as previously described (Granberg et al., 1998). Briefly, tissue slides were prepared about one centimeter thick and fixedin 10% formalin. Then, prepared slides were stained by hematoxylin and eosin method (Miettinen &Jerzy., 2001; Munday et al., 2006; Teifke &Löhr., 1996),to considering pulmonary adenomatosis. Totally, fifteen samples from fifty collected samples were recognized as pulmonary adenomatosis. Finally, immunohistochemistry method (Miettinen &Jerzy., 2001; Munday et al., 2006; Teifke & Löhr., 1996; Mohamadzadeh et al., 2014) was carried out on 15 detected samples and immune stained to study mutation of p53 tumor suppressor gene. Eleven from fifteen samples were positive and referred to molecular analysis.

Isolation of DNA from lung tissue:

DNA was extract on using Accu Prep® Genomic DNA Extraction Kit. Briefly, lung samples were homogenized (25-50 mg) with a mortar and pestle. After we placed homogenized samples in a clean 1.5 ml tube, we added 200 µl of Tissue Lysis buffer (TL). For digestion of the proteins we added 20 µl of Proteinase K, mixed by vortex mixer, and incubated at 60℃until the clarity was changed from turbid to clear. Then spanned down the tube to remove drops from the inside of the lid, added 200 µl of Binding buffer (GC), mixed by vortex mixer, and Incubated at 60℃for 10 min. After this step we Added 100 µl of Isopropanol, mixed well by pipetting and briefly spanned down to get the drops clinging under the lid. the lysate Carefully was transferred into the upper reservoir of the Binding column tube (fit in a 2 ml tube) without wetting the rim, and we centrifuged at 8,000 xg for 1 min. the tubes were Opened and the Binding column tubes were transferred to new 2 ml tube for filtration.

After this step we added 500 µl of Washing buffer 1 (W1) without wetting the rim , it centrifuged at 8,000 xg for1 min. then the tubes were Opened and the solutions were poured from the 2 ml tube into a disposal bottle. Again Carefully we added 500 µl of Washing buffer 2 (W2) without wetting the rim and centrifuged at 8,000 xg for 1 min. for completely remove ethanol we centrifuged once more at ca. 12,000 xg for 1 min. the Binding column tubes were transferred to a new 1.5 ml tube for elution (supplied),then we added 200 µl of Elution buffer on to Binding column tubes, and we waited for at least 1 min at RT (15-25℃) until Elution buffer was completely absorbed into the glass fiber of Binding column tube. In The final step the tubes were centrifuged at 8,000 xg for 1 min to elute. For an improved yield, we eluted the samples twice. Finally, spectrophotometry and agar gel were used to assess the quality and quantity of extracted DNA.

Concentration Determination of the Extracted DNA

Concentration and purity of the extracted DNA was measured using a spectrophotometer (Thermo Scientific Nano Drop™ ND-2000, USA) as previously described (Sambrook et al 1989). The ratio OD_{260}/OD_{280} was used to indicate the purity of the DNAs, where the pure DNA has an OD_{260}/OD_{280} ratio of 1.8 to 2.0. Low ratios could be caused by protein or phenol contamination (Menezes et al., 2002).
DNA samples were also subjected to electrophoresis in an Ethidium Bromide pre-stained 1% (w/v) agarose (CinnaGen, Iran) gel matrix in order to check the DNA quality.

Primers and Polymerase Chain Reaction (PCR)

Two pair primers for 7 & 8 exons of ovine p53 tumor suppressor gene was obtained from previously proposed primers by Dequiedt et al (Dequiedt et al., 1995).Sequencing of primers was as below;
EXONE7:
FORWARD PRIMER: AGGAAACAGGCTCGTGGAGG
REVERSE PRIMER: CGGAGGTGAGGGAAGCTAGC
EXONE8:
FORWARD PRIMER: GGTTCACAGGAGAACACAGA
REVERSE PRIMER: CAGAACTGCACCCTCGCTGC
The extracted DNA was used as template for PCR. The PCR reaction mixture contained 2.5 µl of 10x PCR buffer, 0.5 µl dNTP 10mM, 0.5 µl of forward primer 10 µM, 0.5 µl of reverse primer 10 µM, 1.5 µl of MgCl2 25mM, 0.25 µl of pfu DNA polymerase, 1ul (100-1000ng)of sampleDNA and 18.25 µl of dH_2O to reach final volume to 25ul .
PCR program for the exon 7 was run as follows: After an initial denaturation at 94°C for 5 min, the PCR mixture was subjected to 35 cycles of 20 s at 94°C, 30s at 59°C, 30s at 72°C, and a final extension step at 72°C for 10 min.
PCR program for the exon 8 was run as follows: After an initial denaturation at 94°C for 5 min, the PCR mixture was subjected to 35 cycles of 20 s at 94°C, 30s at 58°C, 30s at 72°C, and a final extension step at 72°C for 10 min.
The PCR products were checked on 1.5% agarose gel (Fermentas, Canada).

Detection and Purification of PCR Products

Amplified products (approximately 6-7ul) were mixed with 2ul of 6X DNA Loading Dye (Fermentas, Canada) and separated on 1.5 % agarose (Fermentas, Canada). The gel was stained in Ethidium Bromide (Sigma, Canada) solution (0.5µg/ml) for 15 min and the expected size of the PCR products were estimated in relation to 100bp DNA ladder (Fermentas, Canada). Then, total amplified fragments of the gene in expected ranges (sharp and specific bands) were purified from the gel using a gel extraction kit (Gene All, Korea) according to the manufacturer's instructions. The quality and quantity of the purified DNA were assessed as dsDNA by spectrophotometer and 1% agarose gel as well. Finally, 30ulpurifiedPCR products were sent to sequencing (Bioneer, South Korea).

RESULTS AND DISCUSSION

In the present study, from 50 recorded lungs in the slaughterhouse through the pathology eleven samples were diagnosed pulmonary adenomatous which accounted for 22% of all of examined lungs. Slides which were identified to be pulmonary adenomatosis positive were colored by immunohistochemistry method, and then were studied carefully under microscope in six slides the brown color that is caused by antibody-antigen complexes were observed. The ratio between the absorbance A260 /A280 was 1/8 to 2, and there was a strong band with high quality and no elongation, it was defined that DNA extraction did well.

PCR amplification of ovine P53 gene

The exons 7 and 8 of ovineP53gene were amplified-polymerase chain reaction (PCR) using the specific primers. To improve the amplified efficiency of the exons, the reactions were performed in a Gradient Thermal Cycler (BioRad, Canada) and the products were analyzed by 1% agarose gel with GeneRuler 100 bp Plus DNA Ladder (Fermentas, Canada) to estimate the size of the PCR products. The PCR products were about 210 base pairs (bp) for the exon 7 and 220 base pairs (bp) for the exon 8, respectively, indicating that the primers and amplification condition were specific (Figures 1 and 2).

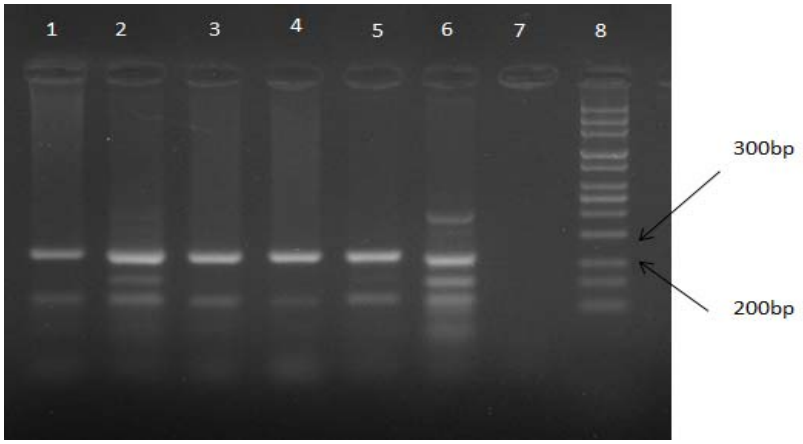

Figure 1: Agarose gel electrophoresis analysis of the PCR product of exon 7 p53 tumors uppressor gene. Lanes 1,2,3,4,5,6 PCR product of exon 7 gene (210bp), lane 7; negative control, lane 8; GeneRuler 100 bp Plus DNA Ladder(Fermentas, Canada).

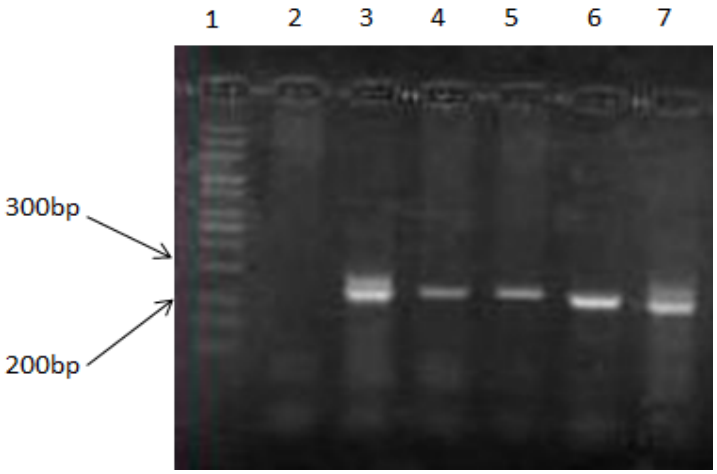

Figure 2: Agarose gel electrophoresis analysis of the PCR product of exon 8 p53 tumor suppressor gene. Lane 1; GeneRuler 100 bp Plus DNA Ladder (Fermentas, Canada), lane 2; negative control, lanes 3,4,5,6,7; PCR product of exon 8 gene (220bp).

Sequences Analysis

Sequencing had done by two pair primers (Forward and Reverse) for each exon. Since fragments size is below 300 base pair whole sequences in exons could identified base to base acutely. All results showed that there were no mutations in exons 7 and 8 of p53 tumor suppressor gene. All samples in the exons 7 and 8of ovine P53 gene had 100% identical alignment with the published sequence of p53 tumor suppressor gene (Accession No: 019468.1).However, our finding showed any mutation within 7 and 8 exons, another study by Olivier et al (2010) shown that high percentage oftp53mutations as single-base substitutions distributed throughout the coding sequence (Olivier et al., 2002). A study by Sanjay Katiyar et al (2000)showed that in hepatocellular carcinoma (HCC) p53 mutations are occur mostly eliminated, penetration, point mutations and more than90% of p53mutationsinbetween codons 110 and 307 in the region containing exons 5 to 8 (Katiyar et al., 2000).Results of present study showed no mutation within 7 and 8 exons. Therefore, our finding is not in line with previously studies.

In this study, immunohistochemical results showed that six of the eleven samples have mutations in the p53 gene, but the results of the PCR products had been sequenced for mutationsinexons7and8revealed that no mutation occurred in these samples. The p53 protein is frequently formed and degraded in the healthy animal's cells. As well as degradation of the p53 protein is related to MDM2 binding. Mutation in p53 proteins often do not stimulate MDM2, as result intent to accumulate at high concentrations in the cells. As a result of immunohistochemicalstainingp53 mutant becomes brown in color due to very high absorption (Matlashewski et al., 1984) whereas, in normal mode, the stability ofp53 was unstable (Bullock et al., 1997).
In this study, we had obtained eleven positive samples (brown color) by immunohistochemistry method but it was not found any mutation. That's reason may be considered in occurred of the mutant in the others exons of p53 exception 7 and 8 exons. In the present study, in the gel electrophoresis of PCR products, there was a non-specific band especially in exon 7. In optimizing of the PCR programs this non-specific band was not removed. It may be due to mismatching of primers to another region of the DNA genomic. We are proposing the exon 7 primers should be to improve in the futures studies. Also, there were extra bands in two samples in exon 8 which was too close to the specific band which Non-specific binding of primers should be consider as a major reason.
In our study, there was one sample that was not amplified by exon8 primers. To solve the problem it was tried to add DNA template in different concentration, used different number of cycles, different annealing temperatures, i.e. but finally the sample was not amplified. That reason may be considered to be mutations in intron because of primers were designed within introns.

A study by Dequiedt et al (1995) showed that Mutations in the p53 tumor-suppressor gene are frequently associated with bovine leukemia virus-induced leukemogenesis in cattle but not in sheep (Dequiedt et al., 1995).This study reports no changes of P53 gene in sheep's pulmonary adenomatosis that it is compatible with Dequiedt et al which noted (Dequiedt et al., 1995).In another study, Mohammad Zadeh et al (2014) reported point mutation in exon 5 and 6of p53tumor-suppressor gene in Iranian sheep related to pulmonary adenomatosis (Mohamadzadeh et al., 2014).However, our study demonstrates that pulmonary adenomatosis disease is not related to mutation exon 7 and 8 ofp53 tumor-suppressor gene.

Bucher et al (1996) by a study showed that the frequency of p53 mutations in equine sarcoid might be low (Bucher et al., 1996).Ueda et al (1995) showed no evidence for p53 mutation in hepatocellular carcinoma, except in advanced tumours where a small proportion of cells may have acquired specific base substitutions (Ueda et al., 1995). Also, Hong-Qi Peng et al (1993) reported Mutations of the p53 gene do not occur in Testis Cancer (Peng et al.,1993). Moreover, Heimdal et al (1993) reposed a similar lack of TP53 mutations in a series of 32 patients with bilateral and familial germ cell tumors (GCT) and 2 patients with sporadic GCT in the germline and in addition 15 tumors lacked TP53 mutations (Lowe et al., 1993). Our results indicated that in the investigated samples of pulmonary adenomatous by positive immunohistochemistry, there are not mutation in exon 7and8 ovine p53 gen. This is first study conducted on exon 7 and 8 of ovine p53 gene in Iranian sheep (Gezel breed). More studies are needed to investigate all exons and introns of ovine p53 tumor suppressor gene in the future.

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Competing interests
The authors have no competing interests with this study.

REFERENCES

Bates S, Phillips AC, Clark PA, Stott F, Peters G, Ludwig RL, Vousden KH. (1998). p14ARF links the tumour suppressors RB and p53. Nature, 395(6698), 124–5.

Brambilla E, Brambilla C.(1997). P53 and lung cancer. PatholBiol (Paris), 45, 852–863.

Bucher K, Szalai G, Marti E, Griot-Wenk ME, Lazary S and Pauli U.(1996). Tumour suppressor gene p53 in the horse: identification, cloning, sequencing and a possible role in the pathogenesis of equine sarcoid. Res Vet Sci, 61 (2), 114-119.

Bullock AN, Henckel J, DeDecker BS, Johnson CM, Nikolova PV, Proctor MR, Lane DP, Fersht AR.(1997). Thermodynamic stability of wild-type and mutant p53 core domain. Proc. Natl. Acad. Sci. U.S.A, 94(26),14338–42

Cirielli C, Corrado T. (1995). Adenovirus-mediated gene transfer of wild-type p53 results in melanoma cell apoptosis in vitro and in vivo. International journal of cancer, 1995, 63.5, 673-679.

Dequiedt F, Kettmann R, Burny A, Willems L.(1995). Mutations in the p53 tumor-suppressor gene are frequently associated with bovine leukemia virus-induced leukemogenesis in cattle but not in sheep. Virology, 209(2), 676-683.

Devilee P, Van Leeuwen IS, Voesten A, Rutteman GR, Vos JH, Cornelisse CJ.(1994). The canine p53 gene is subject to somatic mutations in thypoid carcinoma. Anticancer research, 1994, 14(5A), 2039-2046

Ferreira CG, Tolis C, Giaccone G. (1999). P53 and chemosensitivity. AnnOncol, 10(9):1011-1021

Gilbert, Scott F.(2004). Developmental Biology, 10th ed. Sunderland, MA USA: Sinauer Associates, Inc. Publishers. pp. p.588.

Granberg S, Matts W, IngeJ. (1989). Macroscopic characterization of ovarian tumors and the relation to the histological diagnosis: criteria to be used for ultrasound evaluation. Gynecologic oncology,35.2, 139-144.

Greenblatt MS, Bennett WP, Hollstein M, Harris CC (1994).Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. CancerRes, 54, 4855–4878

Griffiths DJ. Martineau HM,Cousens C. (2010).Pathology and Pathogenesis of Ovine Pulmonary Adenocarcinoma. Journal of Comparative Pathology,142, 260-283.

Gruszka-Westwood AM, Hamoudi RA, Matutes E, Tuset, E, Catovsky, D .(2001). p53 abnormalities in splenic lymphoma with villous lymphocytes. Blood, 97(11), 3552-3558

Hollstein M, Sidransky D, Vogelstein B, Harris CC. (1991). p53 mutations in human cancers. Science, 253(5015),49–53.

Ishiguro N, Furuoka H, Matsui T, Horiuchi M, Shinagawa M, Asahina M, Okada K. (1997). p53 mutation as a potential cellular factor for tumor development in enzootic bovine leukosis. Veterinary immunology and immunopathology,55(4), 351-358.

Johnson A. Sherwood C. Guillermo C, Christopher M. (1998). Weghorst. Mutation of the p53 tumor suppressor gene in spontaneously occurring osteosarcomas of the dog. Carcinogenesis 1998, 19.1, 213-217.

Katiyar S, Dash BC, Thakur V, Guptan RC, Sarin SK., & Das B C. (2000). P53 tumor suppressor gene mutations in hepatocellular carcinoma patients in India. Cancer, , 88(7), 1565-1573.

Kern SE, Kinzler KW, Bruskin A, Jarosz D, Friedman P, Prives C, Vogelstein B.(1991). Identification of p53 as a sequence-specific DNA-binding protein. Science, 252(5013), 1708–11

Lee CH, Ji HL, Dae YK. (2004). Mutation and over expression of p53 as a prognostic factor in canine mammary tumors. Journal of Veterinary Science, 5.1, 63-69.

Lin D, Shields MT, Ullrich SJ, Appela E, Mercer WE. (1992). Growth arrest induced by wild-type p53 protein blocks cells prior to or near the restriction point in late G1 phase. Proceedings of the National Academy of Science(Washington), 89, 9210–9214

Lowe SW, Ruley HE, Jacks T, Housman DE. (1993). p53-dependent apoptosis modulates the cytotoxicity of anticancer agents. Cell, 74(6), 957-967.

Matlashewski G, Lamb P, Pim D, Peacock J, Crawford L, Benchimol S. (1984). Isolation and characterization of a human p53 cDNA clone: expression of the human p53 gene. EMBO J, 3(13), 3257–62

Menezes CC, VantoaiT, McDonald MB, Sediyama T.(2002). Sequence-characterized amplified region (SCAR) technique used for variety discrimination in vinca (Catharanthusroseus (L.) G. Don). RevistaBrasileira de Sementes, 24(1), 299-305.

Miettinen M, and Jerzy L. (2001). Gastrointestinal stromal tumors–definition, clinical, histological, immunohistochemical, and molecular genetic features and differential diagnosis. VirchowsArchiv, 438.1, 1-12.

Mohamadzadeh P, Pejman M and Iraj S. (20014). P53 muiation in ovine pulmonary adenomatosis. Indian J. Sci. Res, 7.1 ,606-617

mostafaK, irajk,mohamadRM.(2013). Evaluation of PCR for Jaagsiekte Sheep Retrovirus Detection from Iranian Sheep Flocks. Global Veterinaria, 10 (1), 84-89,

MundayJS, Brennan MM, &Kiupel M. (2006). Altered Expression of β-catenin, E- cadherin, Cycloxygenase-2, and p53 Protein by Ovine Intestinal Adenocarcinoma Cells. Veterinary Pathology Online, 43(5), 613-621.

Muto T,Wakui S, Takahashi H, Maekawa S, Masaoka T, Ushigome S, Furusato, M. (2000). p53 gene mutations occurring in spontaneous benign and malignant mammary tumors of the dog. Veterinary Pathology Online,37.3,248-253.

National Center for Biotechnology Information. United States National Institutes of Health. The p53 tumor suppressor protein [Retrieved 2008-05-28].

Olivier M., Eeles R, Hollstein M, Khan MA, Harris CC, HainautP. (2002). The IARC TP53 database: new online mutation analysis and recommendations to users. Human mutation, 19(6), 607-614.

Peng HQ, Hogg D, Malkin D, Bailey D, Gallie BL, Bulbul M, GossPE.(1993). Mutations of the p53 gene do not occur in testis cancer. Cancer research, 53(15), 3574-3578.

Rezazadeh F, Zarrini GH, CousensCH, Attarilar N. (2012). Prevalence of jaagsiekte sheep retrovirus infection in North-West Iran. Global Veterinaria, 9, 535-540.

Sambrook, J., & Russell David, W. (1989). Molecular cloning: a laboratory manual. Vol.3. Cold spring harbor laboratory press.

Scobie L, Jackson ME, & Campo MS. (1997). The role of exogenous p53 and E6 oncoproteins in in vitro transformation by bovine papillomavirus type 4 (BPV-4): significance of the absence of an E6 ORF in the BPV-4 genome. Journal of general virology,78(11), 3001-3008.

Sharp J.M and Martini. A.J. (2003). Natural history of JSRV in sheep. Current Topics in Microbiology and Immunology, 275, 55-97.

TeifkeJP andLöhr CV. (1996). Immunohistochemical detection of P53 overexpression in paraffin wax-embedded squamous cell carcinomas of cattle, horses, cats and dogs. Journal of comparative pathology,114.2, 205-210.

Tustin, RC.(1996). Ovine jaagsiekte. J. Sout Afr. Vet. Med. Assoc, 40, 2-23

Ueda H, Ullrich SJ, Gangemi JD, Kappel CA, Ngo L, Feitelson MA, Jay G. (1995). Functional inactivation but not structural mutation of p53 causes liver cancer. Nature genetics, 9(1), 41-47.

Veldhoen N, Stewart J, Brown R, & Milner J. (1998). Mutations of the p53 gene in canine lymphoma and evidence for germ line p53 mutations in the dog. Oncogene, 16(2), 249-255.

Wakui S, Muto T, Yokoo K., Yokoo R, Takahashi H, Masaoka T, Furusato M.(2000). Prognostic status of p53 gene mutation in canine mammary carcinoma. Anticancer research, 21(1B), 611-616.

Ziemer MA, Mason A, Carlson DM.(1982).Cell-free translations of proline-rich protein mRNAs. J. Biol. Chem, 257(18),11176–80

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