RESEARCH ARTICLE

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Alterations in *p53* Influence hTERT, VEGF and MMPs Expression in Oral Cancer Patients

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Abstract

Background: Mutant *p53* is the crucial molecule in the etiopathogenesis of oral cancer. Therefore, we aimed to evaluate the impact of alterations of the *p53* gene and its negative feedback regulator, *MDM2*, on the expression of hTERT, VEGF, and MMPs; the critical genes involved in oral cancer progression. **Material and methods:** *p53* and *MDM2* genotyping were done by PCR-RFLP. *p53* mutation analysis was performed using PCR-SSCP and sequencing. hTERT, VEGFA isoforms, MMP2, and MMP9 mRNA levels were analyzed by semi-quantitative Reverse Transcriptase PCR. **Results:** Arg allele at *p53* exon 4 was significantly associated with overexpression of hTERT, MMP2, and MMP9 individually. Expression of hTERT, VEGF A isoforms, MMP2 and MMP9 were significantly altered in the presence of *p53* and *MDM2* polymorphisms and *p53* mutations in a specific combination. Mutant p53, Arg allele at p53 exon 4 locus, and G/G/or T/T genotype at *MDM2*revealed increased expression of hTERT, VEGF A isoforms, and MMP2/9. **Conclusion:** This study provides evidence that apart from mutant *p53*, naturally occurring sequence variants in *p53*codon 72 (Arg72Pro) (rs1042522) and *MDM2* (rs2279744) significantly alter the expression of hTERT, VEGF-A isoforms, and MMP2/9 in a specific combination. The differential interaction of codon 72 variants with *MDM2*, hTERT, VEGF-A isoforms and MMP2/9 play a role in the aggressiveness of oral cancer. The results have important implications for oral cancer progression and should be explored for innovative treatment options.

Keywords: *p53*- *MDM2*- hTERT- VEGFA- MMPs

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Introduction

Oral cancer ranks among the top three cancers in India (Sung et al., 2021). It has become a health priority because of the very low 5-year survival rate despite the advances in surgery, radiotherapy, and chemotherapy (Le Campion et al., 2017). Among the numerous genetic events that alter normal functions of oncogenes and tumor suppressor genes (TSG) in oral carcinogenesis, *p53* gene deregulation is a very crucial event (Ragos et al., 2018). The presence of somatic mutations and polymorphic features of this TSG gene contributes to altered normal *p53* functions and impacts susceptibility to cancer (Naccarati et al., 2012). Mutant *p53* with its dominant-negative activity and gain of oncogenic functions endows cancer cells with growth advantages and adds complexity to the tumor biology. *p53* lies at the hub of a vast signaling network (Aylon et al., 2016). Although canonical *p53*-mediated tumor suppression is strictly related to cell cycle arrest and apoptosis, accumulatory evidence highlights the involvement of mutant forms of *p53* in processes such as invasion, metastasis, and angiogenesis (Amelio et al., 2018). Studies have reported that mutant *p53* affects the expression of the key molecules; hTERT, VEGFA, MMP2, and MMP9 in various malignancies involved in immortalization, angiogenesis, invasion, and metastasis (Khromova et al., 2009; Yoshioka et al., 2012; Wang et al., 2013; Hong et al., 2014; Liu et al., 2014; Pfister et al., 2017; Payehghadr et al., 2018; Mantovani et al., 2019).

p53 polymorphism might individually modulate cancer risk or interact with polymorphism of its regulator, *MDM2*, or mutations in *p53* and thereby affect the expression of other downstream effectors of *p53* directly or indirectly (Lieschke et al., 2019). *p53* polymorphisms, specifically *p53* Arg72Pro at codon 72 in exon 4 (rs1042522) affect the structure as well as biochemical and biological activities of *p53* proteins (Ozeki et al., 2011). Understanding the comprehensive impact of *p53* alterations (mutation and polymorphism) in the tumor microenvironment is limited. Hence, the current investigation aimed to determine the comprehensive effect of *p53* polymorphism (rs1042522) and mutations as well as *MDM2* polymorphism (rs2279744) on the expression of hTERT, VEGFA

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isoforms (189,183,165,121), MMP2 and MMP9; the critical genes involved in oral cancer progression.

Materials and Methods

Subjects

The study was approved by the Institutional Ethics committee (no. EC/35/2012). Informed consent was obtained from all the participants. A total of 67 histopathologically confirmed and previously untreated oral cancer patients were enrolled in the study excluding patients having major illnesses in the recent past. Demographic details including age, sex, and tobacco habits 4 were collected by administering a detailed questionnaire. Other details for clinico-pathological parameters, like, differentiation, grade, stage, and lymph-node involvement were collected from the hospital records. Demographic and clinicopathological details are mentioned in Table 1.

Specimen collection and processing

Five milliliters of blood was drawn by venipuncture from all the subjects. White Blood Cells were separated and stored at -800C until analysis. Tissue samples were collected at the time of surgery, washed with sterile phosphate buffer saline (pH:7.4), and stored in an RNA stabilizing reagent (Qiagen, USA) at -800C until analyzed.

DNA and RNA isolation

DNA was isolated from peripheral lymphocytes and malignant tissues using commercially available DNA blood mini kit and DNA mini kit (Qiagen, USA), respectively following the manufacturer's instructions. All the DNA samples were quantified using a spectrophotometer (Shimadzu UV-1800, Japan).

RNA isolation was carried out from malignant tissues using RNAeasy mini kit (Qiagen, USA) following the manufacturer's instructions and stored at -800C until analysis. All the RNA samples were quantified using a spectrophotometer (Shimadzu UV-1800, Japan).

Genotyping of p53 (Arg72Pro, rs1042522) and MDM2 (SNP 309 T>G, rs2279744) polymorphism

Genotyping of *p53* (rs1042522) and *MDM2* (rs2279744) polymorphisms was performed by the Polymerase Chain Reaction - Restriction Fragment Length Polymorphism (PCR-RFLP) method as mentioned earlier (Yu et al., 2011; Patel et al., 2013).

p53 mutation analysis

Mutation analysis was carried out on genomic DNA isolated from malignant tissues of oral cancer patients by Polymerase Chain Reaction –Single-Strand Conformation Polymorphism (PCR-SSCP) covering exons 4-9 of the *p53* gene followed by DNA sequencing as mentioned earlier (Singh et al., 2015).

Reverse transcription-polymerase chain reaction (RT-PCR) for hTERT, VEGFA isoforms, MMP2 and MMP9

One-step RT-PCR was carried out using one-step RT-PCR kit (Qiagen, USA) and primers (Integrated DNA Technologies, USA) as mentioned earlier (Patel et al., 2015). *β-actin* was used as an internal control.

PCR products were run on 1.5% ethidium bromide-stained agarose gel for hTERT, MMP2, and MMP9. For VEGFA isoforms, PCR products were run on 6% native polyacrylamide gel and visualized after staining with ethidium bromide. The image was captured and analyzed by a gel documentation system (Alpha Innotech, USA). The band intensity of hTERT, VEGFA isoforms, MMP2 andMMP9 and was quantified along with the band intensity of their respective *β-actin* expression. hTERT, VEGFA isoforms, MMP2, and MMP9 to *β-actin* ratio were calculated to find out the expression index of hTERT, VEGFA isoforms, MMP2 and MMP9.

Statistical analysis

Statistical analysis was carried out using SPSS (Version 20) software. Transcripts levels were expressed as Mean ± Standard Error of Mean (SEM). The samples were analyzed in duplicates. An independent "t" test was carried out to compare mRNA levels with *p53* and *MDM2* genotypes as well as *p53* mutation status. A Chi-square test was utilized to look for an association between genotypes and mutations. $p<0.05$ was considered statistically significant.

Results

Frequency distribution of the genotypes (p53 exon 4, rs1042522; MDM2, rs2279744) and mutations in the study subjects

The frequency distribution pattern of *p53* exon 4 genotypes revealed a higher prevalence of Arg/Arg (37.3%; 25/67) followed by Arg/Pro (34.3%; 23/67) and Pro/Pro (28.4%; 19/67). The genotyping of *MDM2* revealed a higher percentage of individuals with heterozygous variants (G/T; 52.0%; 35/67) followed by homozygous TT (25.4%; 17/67) and G/G (22.4%; 15/67).

Sequencing confirmed the mutations in malignant tissues of 26 (39.1%) cases. (19; 72.2%) of the mutations were missense type and 7(27.8%) were truncating type mutations.

Association of p53 exon 4 (rs 1042522) and MDM2 (rs2279744) genotypes with p53 mutations

We looked for evidence of an association between *p53* and *MDM2* genotypes with *p53* mutations. No significant association was observed. However, the presence of *p53* mutations was higher in cases with T/T genotype. Interestingly, cases with G/G genotype showed lower mutation frequency (Supplementary FigureA 1).

Association between p53 alterations, MDM2 polymorphism, and hTERT gene expression

hTERT transcript levels were higher in cases with the Arg allele at exon 4 of *p53* (Arg/Arg vs Pro/Pro; p=0.032; Figure 1A). However, *p53* mutations and *MDM2* rs2279744 did not significantly affect hTERT mRNA levels (Figure 1B and 1C).

The statistical analysis for combined data revealed that cases homozygous for the Arg allele and *p53* mutation

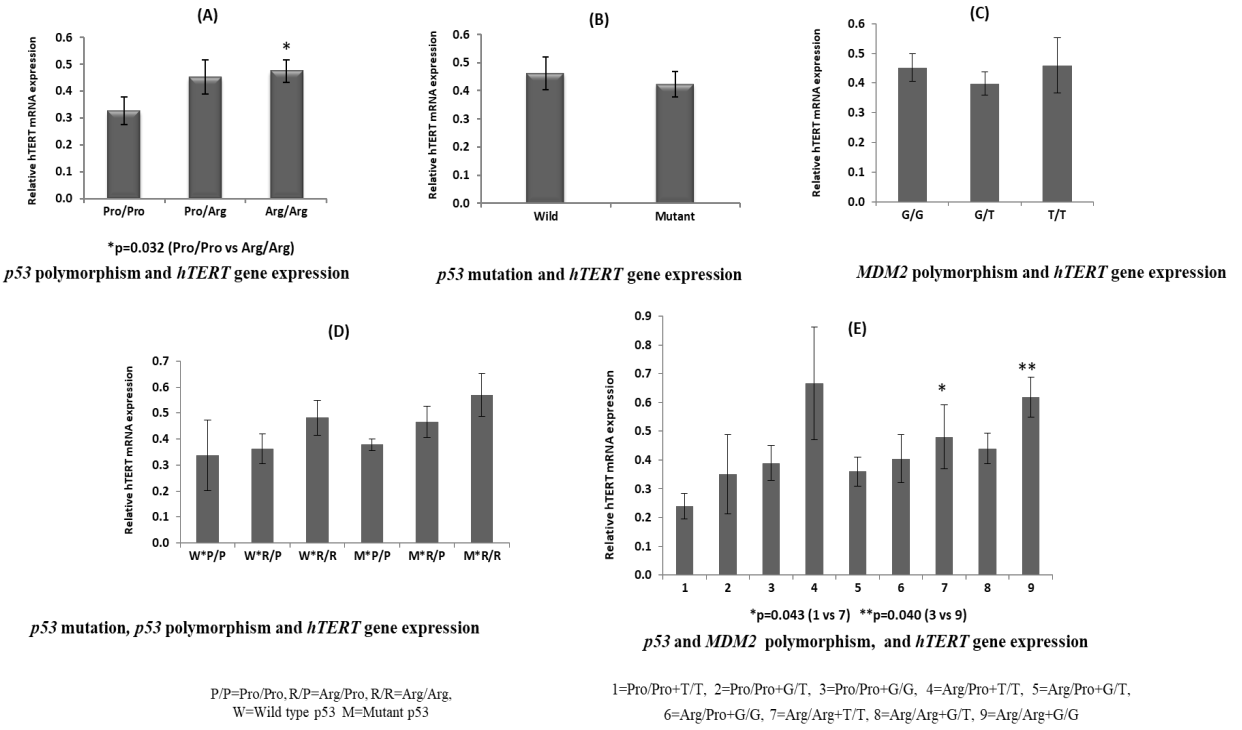


Figure 1. Association between *p53* Alterations, *MDM2* Polymorphism and *hTERT* Gene Expression. (A) *p53* polymorphism and *hTERT* gene expression; (B) *p53* mutation and *hTERT* gene expression; (C) *MDM2* polymorphism and *hTERT* gene expression; (D) *p53* mutation, *p53* polymorphism and *hTERT* gene expression; (E) *p53* and *MDM2* polymorphism, and *hTERT* gene expression. P/P, Pro/Pro; R/P, Arg/Pro; R/R, Arg/Arg; W, Wild type *p53* M, Mutant *p53*; 1, Pro/Pro+T/T; 2, Pro/Pro+G/T; 3, Pro/Pro+G/G; 4, Arg/Pro+T/T; 5, Arg/Pro+G/T; 6, Arg/Pro+G/G; 7, Arg/Arg+T/T; 8, Arg/Arg+G/T; 9, Arg/Arg+G/G

had higher hTERT gene expression compared to the cases homozygous for the Pro allele (Figure 1D). In contrast, *MDM2* polymorphism (rs2279744) and *p53* mutations together failed to affect hTERT transcript levels. Cases with Arg/Arg genotype (rs1042522) and homozygous for either T/T or G/G for *MDM2* (rs2279744) had significantly higher hTERT mRNA levels than cases with Pro/Pro (rs1042522) genotype with either T/T or G/G for *MDM2* (rs2279744) (p=0.043 and p=0.040, respectively) (Figure 1E).

Association between p53 alterations, MDM2 polymorphism, and expression of VEGF A isoforms

The analysis for the association between *p53* and *MDM2* polymorphism on VEGFA isoforms did not reveal any significant data (Supplementary Figure A.2).

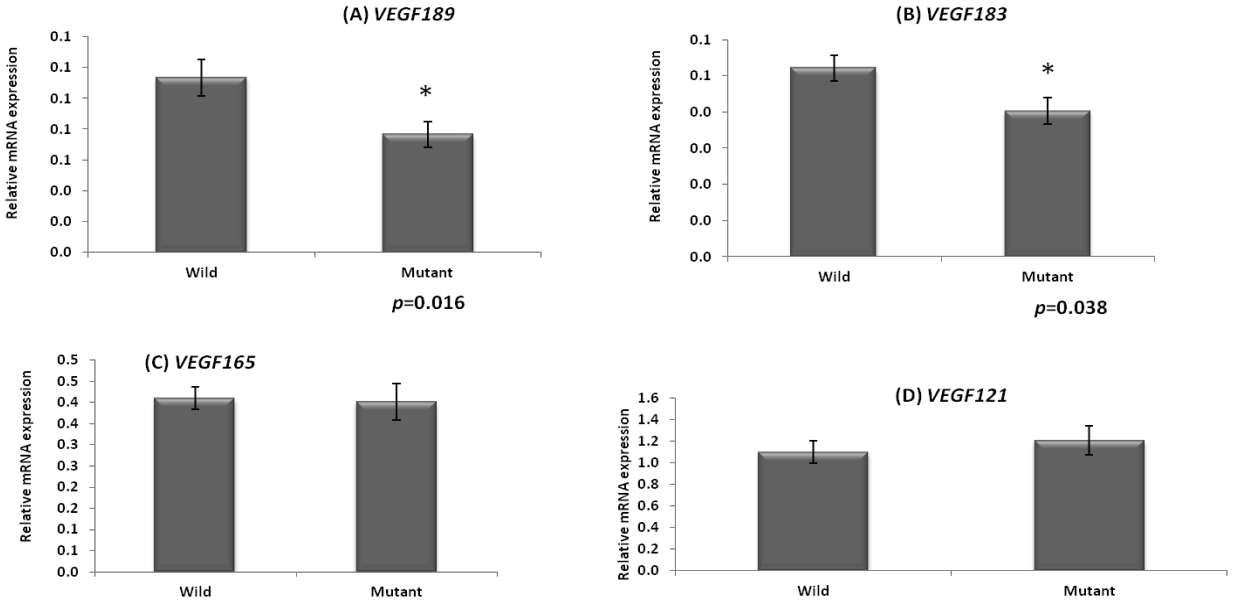


Figure 2. Association between *p53* Mutations and Transcript Levels of VEGFA Isoforms

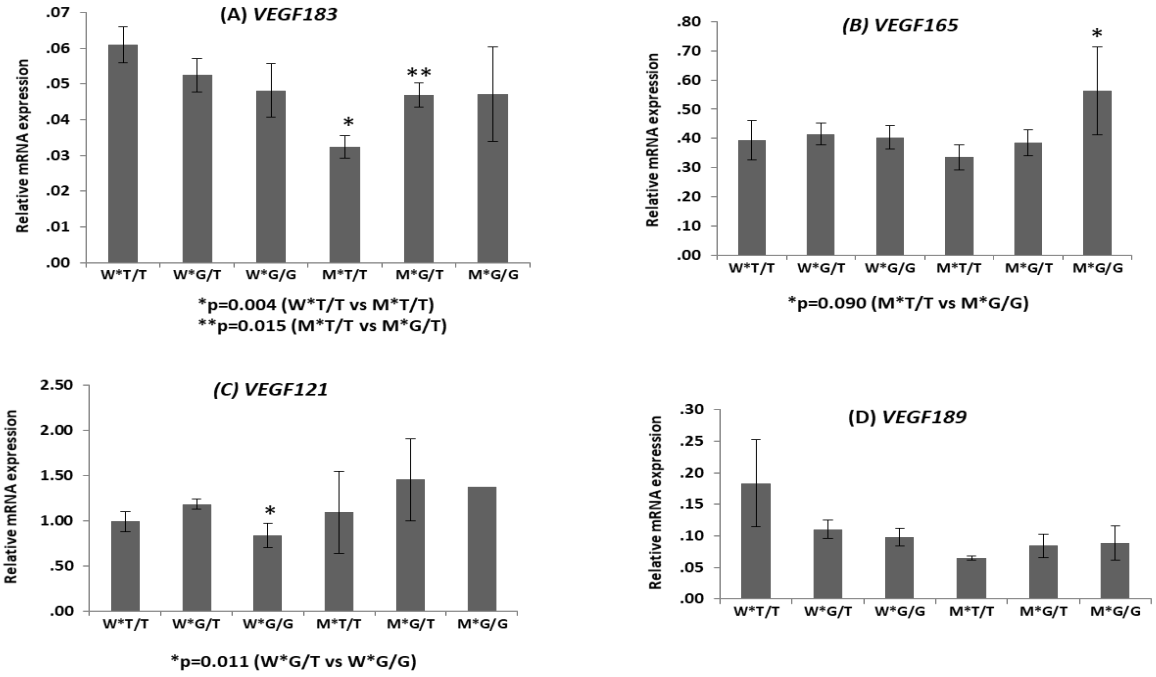
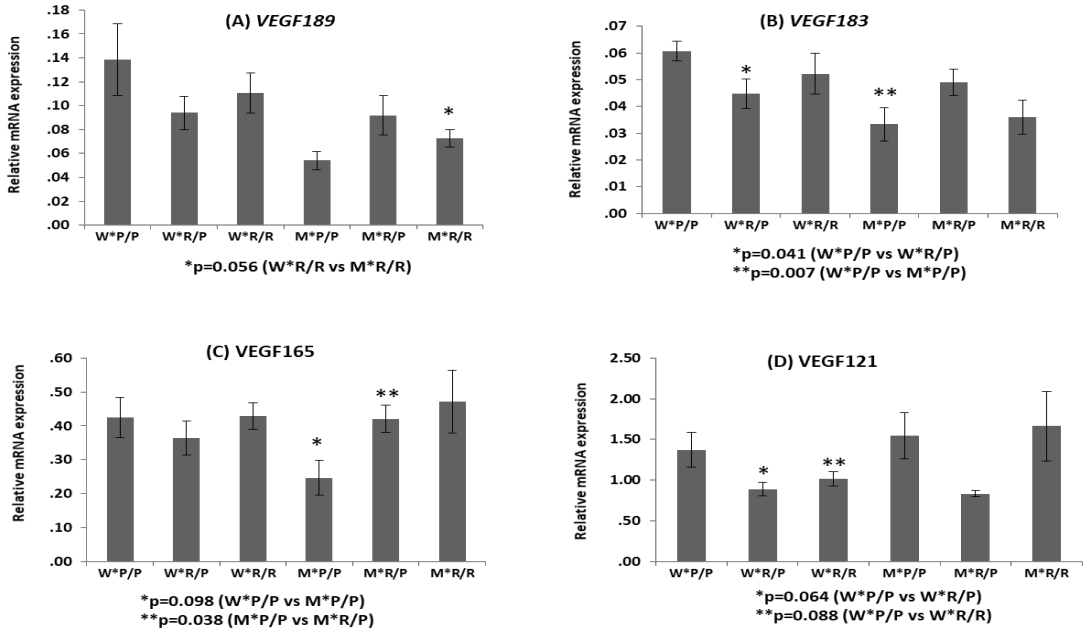


Figure 3. Association of Transcript Levels of VEGFA Isoforms with *p53* Exon 4 Genotypes and Mutations (in combination). P/P, Pro/Pro; R/P, Arg/Pro; R/R, Arg/Arg; W, Wild type *p53*; M, Mutant *p53*.

However, the presence of mutations lead to a significant decrease in the expression of VEGF 189 and VEGF 183 isoforms (p=0.016 and p=0.038; Figure 2A and 2B).

Further with combined analysis, the VEGF 189 mRNA levels were found to be lower in tumors with Arg/Arg genotype and mutant *p53* in comparison to tumors with wild-type *p53* and similar genotype (p=0.056; Figure 3A). VEGF183 transcript levels were significantly higher in patients with Pro/Pro genotype compared to patients with Arg/Pro genotype at *p53* exon 4 (rs1042522) in combination with wild-type *p53*, (p=0.041, Figure 3B). Interestingly, VEGF183 transcript levels were lower in tumors with mutant *p53* as compared to tumors with wild-type *p53* in combination with the Pro/Pro genotype at *p53* exon 4 locus (p=0.007; Figure 3B). Also, VEGF165 transcript levels were significantly higher in patients with Arg/Pro genotype as compared to patients with Pro/Pro genotype at *p53* exon 4 in combination with mutant *p53* (p=0.038, Figure 3C). Intriguingly, VEGFA isoform expression was higher in cases with mutant *p53* and Arg allele (Figure 3).

The results for combined analysis with mutant *p53*



P/P=Pro/Pro; R/P=Arg/Pro; R/R=Arg/Arg; W=Wild type *p53*; M=Mutant *p53*

Figure 4. Association of Transcript Levels of VEGFA Isoforms with *MDM2* SNP309 Genotypes and *p53* Mutations (in combination). W, Wild type *p53*; M, Mutant p53.

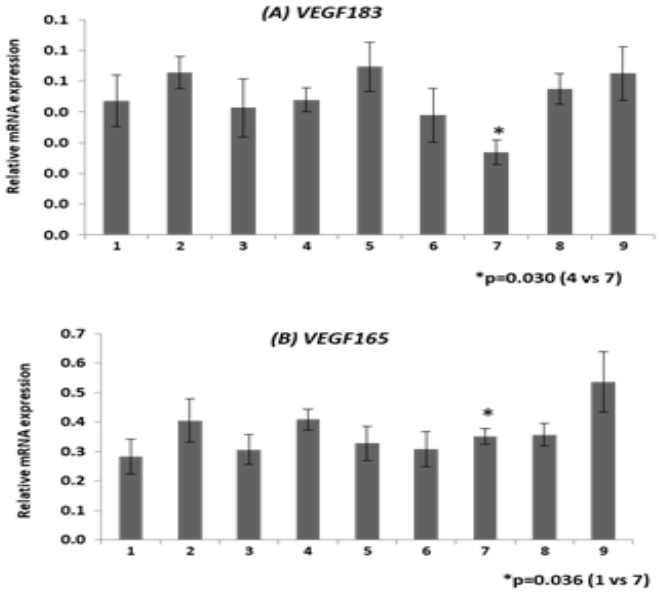


Figure 5. Association of Transcript Levels of VEGFA Isoforms with *p53* Exon 4 and *MDM2* SNP309 Genotypes (in combination). 1, Pro/Pro+T/T; 2, Pro/Pro+G/T; 3, Pro/Pro+G/G; 4, Arg/Pro+T/T; 5, Arg/Pro+G/T; 6, Arg/Pro+G/G, 7, Arg/Arg+T/T, 8, Arg/Arg+G/T; 9, Arg/Arg+G/G

and *MDM2* (rs2279744) suggested that mutant *p53* and G/T or G/G genotype at *MDM2* locus was associated with higher VEGF A isoform expression levels as compared to patients with T/T genotype (Figure 4). Interestingly, patients with Arg/Pro genotype at exon 4 and T/T genotype at *MDM2* exhibited significantly higher mRNA levels of VEGF 183 as compared to patients homozygous for Arg allele (Arg/Arg) with T/T genotype (p=0.030) (Figure 5A). Similarly, higher VEGF 165 mRNA levels were observed in cases with Arg/Arg and T/T genotype than cases with Pro/Pro and T/T genotype for *p53* exon 4 and *MDM2* polymorphism, respectively (p=0.036) (Figure 5B).

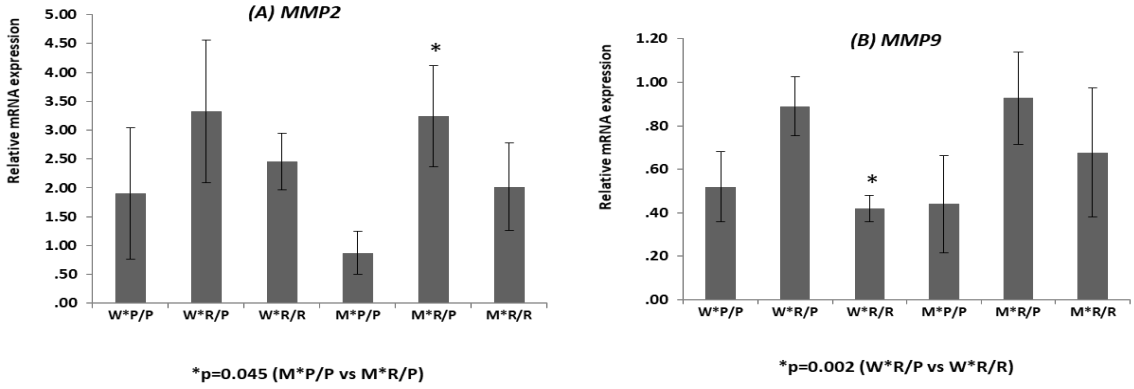


Figure 6. Association of MMP2 and MMP9 Transcript Levels with (A) *p53* Genotypes and (B) MDM2 Genotypes

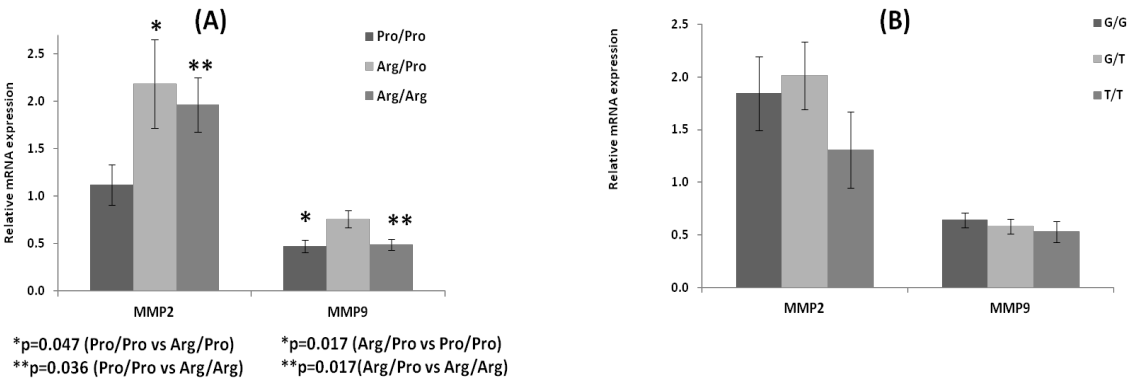


Figure 7. Association of MMP2 and MMP9 Transcript Levels with *p53* Exon 4 Genotypes and p53 Mutations (in combination). W, Wild type *p53*; M, Mutant *p53*

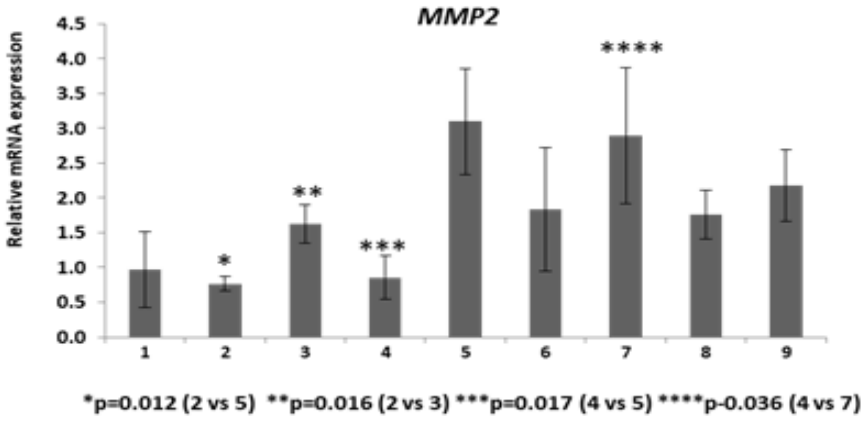


Figure 8. Association of MMP2 Transcript Levels with *p53* Exon 4 and MDM2 SNP309 Genotypes (in combination). 1, Pro/Pro+T/T; 2, Pro/Pro+G/T; 3, Pro/Pro+G/G; 4, Arg/Pro+T/T; 5, Arg/Pro+G/T; 6, Arg/Pro+G/G; 7, Arg/Arg+T/T; 8, Arg/Arg+G/T; 9, Arg/Arg+G/G

Association between p53 alterations, MDM2 polymorphism, and MMP-2 and MMP-9 gene expression

The association analysis revealed that *p53* polymorphism (rs1042522) significantly affected MMP-2 expression levels (Figure 6A). Heterozygous (Arg/Pro) and homozygous patients (Arg/Arg) for *p53* exon 4 had significantly elevated MMP2 transcript levels as compared to homozygous patients (Pro/Pro) (p=0.047 and p=0.036, respectively). Also, lower MMP-9 transcript levels were seen in patients homozygous for Arg or Pro allele (p=0.017 and p=0.017; Figure 6A). However, *MDM2* polymorphism (Figure 6B) and *p53* mutations (Supplementary Figure A.3) did not show any significant association.

The results for the combination approach were; higher MMP-2/9 mRNA levels in cases with Arg allele and *p53* mutation than in cases with Pro/Pro genotype at exon 4 locus with mutation (Figure 7). Further, the combined analysis of MMP-2 expression levels with *p53*mutations and *MDM2* (rs2279744) polymorphism yielded no clear trend. (Supplementary Figure A.4). MMP-2 expression levels were significantly higher in patients with Arg/Arg genotype compared to patients with Arg/Pro genotype at exon 4 locus with T/T genotype at *MDM2* locus (p=0.036) (Figure 8).

Table 1. Demographic and Clinico-pathological Parameters of the Cases

Clinico-pathological Characteristics (N=67)	No. (%)
Sex: Male	58 (86.6)
Female	09 (13.4)
Age: Male - Mean (Range)	(47.7) 28 - 75
Female - Mean (Range)	(44.8) 22 - 55
Both - Mean (Range)	(47.3) 22 - 75
Tobacco habits: Tobacco non-habituates	08 (11.9)
Tobacco habituates	59 (88.1)
Histopathology: Oral squamous cell carcinoma	67 (100)
Site: Buccal mucosa	31 (46.3)
Tongue	13 (19.4)
Alveolus	06 (9.0)
Lips	03 (4.5)
Others	05 (7.5)
Multiple sites	09 (13.4)
Tumor Differentiation: Well	25 (37.3)
Moderate	34 (50.7)
Poor	04 (6.0)
Not Available	04 (6.0)
Tumor Size: Small < 4 cms	36 (53.7)
Large ≥ 4 cms	30 (44.8)
Not Available	01 (1.5)
Stage: Early [Stage I + Stage II]	24 (35.8)
Advanced [Stage III + Stage IV]	42 (62.7)
Not Available	01 (1.5)
Lymph Node Metastasis: Non - Metastasis	38 (56.7)
Metastasis	29 (43.3)
Mode of Invasion: Localized	25 (37.3)
Invasive	40 (59.7)
Not Available	02 (3.0)

Discussion

In the present study, we made a comprehensive analysis of the mechanisms by which the normal function of *p53* is affected and altered and how the altered *p53* affects the expression of other genes involved in various hallmarks of cancer i.e. immortalization (hTERT), angiogenesis (VEGFs) and invasion and metastasis (MMPs).

Association of p53 alterations, MDM2 polymorphism with hTERT transcript levels

Lower levels of hTERT mRNA have been reported in normal mucosa with a gradual increase during malignant transformation (Hrstka et al., 2009). Transcription of hTERT has been shown to be downregulated following the induction of *p53* (Cukusić et al., 2008). hTERT gene has two *p53* binding motifs upstream of the 5′ core promoter region. Overexpression of *p53* and its subsequent binding to these two motifs with the help of transcription factor Sp1 leads to the repression of the hTERT promoter

(Lai et al., 2007). In our study, *p53* exon 4 polymorphism was significantly associated with hTERT mRNA expression individually, though no such association was seen with *MDM2* polymorphism and *p53* mutation status. A number of studies have reported a positive correlation between hTERT mRNA and *p53* protein expression in various malignancies through immunohistochemistry (Dai et al., 2001; Tang et al., 2006). However, looking only at *p53* protein expression could be misleading to draw any conclusion on the status of *p53* alterations. It is reported that several mutations can completely abrogate its function (Freed-Pastor et al., 2012; Yamamoto et al., 2018) and polymorphisms affect the structure as well as functional activities of *p53*. To the best of our knowledge, there is no data regarding the association of hTERT expression with *p53* and *MDM2* polymorphisms. When combined analysis of *p53* polymorphisms, *p53* mutations and hTERT expression was performed, it was observed that hTERT expression was increased in cases with Arg/Arg genotype as compared to cases with Pro/Pro genotype in combination with *p53* mutations as well as in combination with either G/G and T/T genotypes of*MDM2*. Arg allele is reported to have a greater capacity to interact with MDM-2 resulting in enhanced ubiquitination (Hrstka et al., 2009), and increased hTERT expression. Mutations might affect the DNA binding domain of *p53* which is required for the repression of hTERT, however, no direct binding of *p53* to the hTERT core promoter has been reported (Ramlee et al., 2016).

Association of p53 alterations, MDM2 polymorphism with VEGFA isoform transcript levels

p53 is shown to be intimately involved in the process of neo-vascularization often through various inhibitory mechanisms (Li et al., 2020). New evidence suggests that regulation of VEGF promoter by *p53* is more complex than simply indirectly repressing VEGF expression by interaction and inhibition of transcription factors such as SpI and E2F (Farhang Ghahremani et al., 2013). *p53* regulates the expression of VEGFA through hypoxia-inducible factors-1α (HIF-1α) (Farhang Ghahremani et al., 2013). Hypoxia is an important angiogenic switch, critical for the growth of solid tumors. *p53* responds to hypoxic stress, a potential functional crosstalk happens between these two key molecules, the mechanism of which is unclear (Skirnisdottir et al., 2016). A recent study has documented an interaction between *p53* mutant and its regulator for subunit of HIF-1α, this transcriptional complex has been implicated in the regulation of VEGFA (Amelio et al., 2018).

There are contradictory studies in the literature on the effect of *p53* mutations on VEGFA, several authors have suggested upregulation and others have reported no association (Cho et al., 2007; Soussi et al., 2007; Khromova et al., 2009). The discrepancy between these results might be explained by: (i) differences in the methods used to assess *p53* mutation and VEGFA expression in cancer tissues, (ii) the antibodies used, iii) the patient populations, and (iv) no simultaneous analysis of *p53* and *MDM2* polymorphism. In several studies, VEGFA expression was assessed by IHC, which is frequently influenced by tissue preparation and antibodies used (Yuan et al., 2002). We found various VEGA isoforms which were significantly lower in mutant *p53* patients. Moreover, the presence of various VEGFA isoforms in the tissues might influence the association of VEGFA expression and *p53* mutations. However, there is no data regarding the association of VEGFA isoform expression with *p53* mutations and also with *p53* and *MDM2* polymorphisms in the literature.

In the present study, it was observed that VEGFA isoforms i.e.VEGF189, VEGF183, VEGF165, and VEGF121 were significantly altered in the presence of specific combination of *p53* polymorphism and mutations and *MDM2* polymorphism. VEGFA isoforms did not show any association with *p53* and *MDM2* genotypes individually. However, we observed that VEGFA isoforms were lower in patients with mutant *p53*. Intriguingly, VEGFA isoform expression was increased in cases with mutant harboring Arg allele at *p53* exon 4 locus and G allele for *MDM2* than in mutants with Pro allele at *p53* exon 4 locus and T allele for *MDM2*.

Numerous recent studies suggest that VEGFA expression is regulated through *p53*/*MDM2* pathway and other reports suggest *MDM2* regulates VEGFA expression in a *p53* independent way (Rathinavelu et al., 2012; Muthumani et al., 2014; Xiong et al., 2014). However, the findings of the present study support the notion that *MDM2* might regulate VEGFA expression in *p53* dependent manner (Narasimhan et al., 2008).

Association of MMP2 and MMP9 expression with p53 gene status and MDM2 polymorphism

The regulation of MMPs by *p53* is complex, and studies have reported that it upregulates MMP2 but downregulates MMP9 (Powell et al., 2014). There is a paucity of data to document the simultaneous effect of *p53*, *MDM2* polymorphism, and *p53* mutations on MMP2 and MMP9 expression in oral carcinogenesis. In the present investigation, we observed that the presence of Arg allele at *p53* exon 4 locus individually as well as in combination with mutant *p53* resulted into higher MMP2transcript levels. Similarly, the presence of Arg and T allele at *p53* exon 4 and *MDM2*, respectively together resulted into higher MMP2 transcript levels. It suggests that the presence of Arg allele might result into over-expression of MMP2 and this association was altered in the presence of *p53* mutations and *MDM2* polymorphism. MMP9 expression was increased in presence of Arg allele with either mutant or wild-type *p53*.

The results of the current investigation suggest that *p53*, *MDM2* polymorphisms, and *p53* mutations in a specific combination affect transcript levels of hTERT, VEGA isoforms, and MMP2/9 and hence contributes to invasion and metastasis in oral cancer. These results support the findings of recent studies (Basu et al., 2018; Ortiz et al., 2018; Yamamoto et al., 2018) that SNPs may also be an intergenic modifier for gain of function effect of mutant *p53* with Arg variant showing an enhanced invasive and metastatic properties of mutant *p53*. The present investigation highlights the novel role of naturally occurring sequence variants in *p53* gene as well as its

feedback negative regulator, *MDM2* in the regulation of *p53* target genes specifically in oral carcinogenesis. However, the limitations of the present study correspond to relatively limited number of patients included and the method of semi-quantitative analysis used for interpretation.

The study concludes that in addition to oral cancer risk association, *p53* and *MDM2* polymorphism might play an important role in oral carcinogenesis through altered expression of *hTERT, VEGFA, MMP2*, and *MMP9* genes and hence, further contribute to the aggressive behavior of oral cancer. In furtherance, additional evaluation of the clinical significance of these interactions should be done.

Author Contribution Statement

Ragini Singh: conceived and planned the experiments, analysis and interpretation of the results, took the lead in manuscript writing; Kinjal Patel: Planned and carried out the experiments, analysis and interpretation of results, manuscript preparation; Jayendra Patel: analysis and interpretation of results, manuscript preparation; Prabhudas Patel: study conception and design, analysis and interpretation of the results, provided critical feedback and helped shape the research, analysis and manuscript.

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Conflict of interest

The authors declare that they have no conflict of interest.

References

Amelio I, Mancini M, Petrova V, et al (2018). *p53* mutants cooperate with HIF-1 in transcriptional regulation of extracellular matrix components to promote tumor progression. *Proc Natl Acad Sci U S A*, **115**, E10869-78.

Aylon Y, Oren M (2016). The Paradox of *p53*: What, How, and Why?. *Cold Spring Harb Perspect Med*, **6**, a026328.

Basu S, Gnanapradeepan K, Barnoud T, et al (2018). Mutant *p53* controls tumor metabolism and metastasis by regulating PGC-1α. *Genes Dev*, **32**, 230-43.

Cho JH, Kim HS, Park CS, et al (2007). Maspin expression in early oral tongue cancer and its relation to expression of mutant-type *p53* and vascular endothelial growth factor (VEGF). *Oral Oncol*, **43**, 272-7.

Cukusić A, Skrobot Vidacek N, Sopta M, Rubelj I (2008). Telomerase regulation at the crossroads of cell fate. *Cytogenet Genome Res*, **122**, 263-72.

Dai D, Zhai W, Zhu T, Zhu H, Ling Y (2001). Expression of telomerase reverse transcriptase and its relationship with tumor suppressor gene *p53* in HCC. *Zhonghua Gan Zang Bing Za Zhi*, **9**, 79-81.

Farhang Ghahremani M, Goossens S, Nittner D, et al (2013). *p53* promotes VEGF expression and angiogenesis in the absence of an intact p21-Rb pathway. *Cell Death Differ*, **20**, 888-97.

Freed-Pastor WA, Prives C (2012). Mutant *p53*: one name, many proteins. *Genes Dev*, **26**, 1268-86.

Hong B, van den Heuvel AP, Prabhu VV, Zhang S, El-Deiry WS (2014). Targeting tumor suppressor *p53* for cancer therapy: strategies, challenges and opportunities. *Curr Drug Targets*, **15**, 80-9.

Hrstka R, Coates PJ, Vojtesek B (2009). Polymorphisms in *p53* and the *p53* pathway: roles in cancer susceptibility and response to treatment. *J Cell Mol Med*, **13**, 440-53.

Khromova NV, Kopnin PB, Stepanova EV, Agapova LS, Kopnin BP (2009). *p53* hot-spot mutants increase tumor vascularization via ROS-mediated activation of the HIF1/VEGF-A pathway. *Cancer Lett*, **276**, 143-51.

Lai SR, Cunningham AP, Huynh VQ, Andrews LG, Tollefsbol TO (2007). Evidence of extra-telomeric effects of hTERT and its regulation involving a feedback loop. *Exp Cell Res*, **313**, 322-30.

Le Campion ACOV, Ribeiro CMB, Luiz RR, et al (2017). Low survival rates of oral and oropharyngeal squamous cell carcinoma. *Int J Dent*, **2017**, 5815493.

Li AM, Boichard A, Kurzrock R (2020). Mutated TP53 is a marker of increased VEGF expression: analysis of 7,525 pan-cancer tissues. *Cancer Biol Ther*, **21**, 95-100.

Lieschke E, Wang Z, Kelly GL, Strasser A (2019). Discussion of some 'knowns' and some 'unknowns' about the tumour suppressor *p53*. *J Mol Cell Biol*, **11**, 212-23.

Liu J, Zhang C, Feng Z (2014). Tumor suppressor *p53* and its gain-of-function mutants in cancer. *Acta Biochim Biophys Sin (Shanghai)*, **46**, 170-9.

Mantovani F, Collavin L, Del Sal G (2019). Mutant *p53* as a guardian of the cancer cell. *Cell Death Differ*, **26**, 199-212.

Muthumani P, Alagarsamy K, Dhandayuthapani S, Venkatesan T, Rathinavelu A (2014). Pro-angiogenic effects of *MDM2* through HIF-1α and NF-κB mediated mechanisms in LNCaP prostate cancer cells. *Mol Biol Rep*, **41**, 5533-41.

Naccarati A, Polakova V, Pardini B, et al (2012). Mutations and polymorphisms in TP53 gene--an overview on the role in colorectal cancer. *Mutagenesis*, **27**, 211-8.

Narasimhan M, Rose R, Ramakrishnan R, Zell JA, Rathinavelu A (2008). Identification of HDM2 as a regulator of VEGF expression in cancer cells. *Life Sci*, **82**, 1231-41.

Ortiz GJ, Lozano G (2018). SNPing away at mutant *p53* activities. *Genes Dev*, **32**, 195-6.

Ozeki C, Sawai Y, Shibata T, et al (2011). Cancer susceptibility polymorphism of *p53* at codon 72 affects phosphorylation and degradation of *p53* protein. *J Biol Chem*, **286**, 18251-60.

Patel KR, Vajaria BN, Begum R, et al (2013). Association between *p53* gene variants and oral cancer susceptibility in population from Gujarat, West India. *Asian Pac J Cancer Prev*, **14**, 1093-1100.

Patel KR, Vajaria BN, Begum R, et al (2015). VEGFA isoforms play a vital role in oral cancer progression. *Tumour Biol*, **36**, 6321-32.

Payehghadr S, Bahrami, N, Mohammadi F, et al (2018). Detection of miR-21, MUC1mRNA and VEGF protein biomarkers expression changes in oral squamous cell carcinomas (OSCC) in peripheral blood. *Asian Pac J Cancer Biol*, **3**, 59-64.

Pfister NT, Prives C (2017). Transcriptional regulation by wild-type and cancer-related mutant forms of *p53*. *Cold Spring Harb Perspect Med*, **7**, a026054.

Powell E, Piwnica-Worms D, Piwnica-Worms H (2014). Contribution of *p53* to metastasis. *Cancer Discov*, **4**, 405-14.

Ragos V, S Mastronikolis N, Tsiambas E, et al (2018). *p53* mutations in oral cavity carcinoma. *J BUON*, **23**, 1569-72.

Ramlee MK, Wang J, Toh WX, Li S (2016). Transcription regulation of the human telomerase reverse transcriptase (hTERT) gene. *Genes (Basel)*, **7**, 50.

Rathinavelu A, Narasimhan M, Muthumani P (2012). A novel

regulation of VEGF expression by HIF-1α and STAT3 in HDM2 transfected prostate cancer cells. *J Cell Mol Med*, **16**, 1750-7.

Singh RD, Patel KR, Patel PS (2016). *p53* mutation spectrum and its role in prognosis of oral cancer patients: A study from Gujarat, West India. *Mutat Res*, **783**, 15-26.

Skirnisdottir I, Seidal T, Åkerud H (2016). The relationship of the angiogenesis regulators VEGF-A, VEGF-R1 and VEGF-R2 to *p53* status and prognostic factors in epithelial ovarian carcinoma in FIGO-stages I-II. *Int J Oncol*, **48**, 998-1006.

Soussi T (2007). *p53* alterations in human cancer: more questions than answers. *Oncogene*, **26**, 2145-56.

Sung H, Ferlay J, Siegel RL, et al (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*, **71**, 209-49.

Tang F, Gu DH, Wang H, et al (2006). Expression and significance of hTERT mRNA in breast carcinoma and its relation to *p53*. *Zhonghua Zhong Liu Za Zhi*, **28**, 192-5.

Wang Y, Zhang YX, Kong CZ, Zhang Z, Zhu YY (2013). Loss of *P53* facilitates invasion and metastasis of prostate cancer cells. *Mol Cell Biochem*, **384**, 121-7.

Xiong J, Yang Q, Li J, Zhou S (2014). Effects of *MDM2* inhibitors on vascular endothelial growth factor-mediated tumor angiogenesis in human breast cancer. *Angiogenesis*, **17**, 37-50.

Yamamoto S, Iwakuma T (2018). Regulators of oncogenic mutant TP53 gain of function. *Cancers (Basel)*, **11**, 4.

Yoshioka Y, Shimizu S, Ito T, et al (2012). *p53* inhibits vascular endothelial growth factor expression in solid tumor. *J Surg Res*, **174**, 291-7.

Yu H, Huang YJ, Liu Z, et al (2011). Effects of *MDM2* promoter polymorphisms and *p53* codon 72 polymorphism on risk and age at onset of squamous cell carcinoma of the head and neck. *Mol Carcinog*, **50**, 697-706.

Yuan A, Yu CJ, Luh KT, et al (2002). Aberrant *p53* expression correlates with expression of vascular endothelial growth factor mRNA and interleukin-8 mRNA and neoangiogenesis in non-small-cell lung cancer. *J Clin Oncol*, **20**, 900-10.