Implication of ARID1A Undercurrents and PDL1, TP53 Overexpression in Advanced Gastric Cancer

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AT-rich interactive domain-containing protein 1A (ARID1A), TP53 and programmed cell death-ligand 1 (PDL1) are involved in several protein interactions that regulate the expression of various cancer-related genes involved in the progression of the cell cycle, cell proliferation, DNA repair, and apoptosis. In addition, gene expression analysis identified some common downstream targets of ARID1A and TP53. It has been established that tumors formed by ARID1A-deficient cancer cells exhibited elevated PDL1 expression. However, the aberrations in these molecules have not been studied in this population especially in Gastric Cancer (GC). In this backdrop we aimed to investigate the role of the ARID1A mutation and expression of ARID1A, TP53 and PDL1 genes in the etiopathogenesis of Gastric Cancer (GC) in the ethnic Kashmiri population (North India). The study included 103 histologically confirmed GC cases. The mutations, if any, in exon-9 of ARID1A gene was analysed by Polymerase Chain Reaction (PCR) followed by Sanger sequencing. The mRNA expression of the ARID1A, TP53 and PDL1 genes was analysed by Quantitative real-time PCR (qRT-PCR). We identified a nonsense mutation (c.3219; C > T) in exon-9 among two GC patients (∼2.0%), which introduces a premature stop codon at protein position 1073. The mRNA expression of the ARID1A, TP53 and PDL1 genes was significantly reduced in 25.3% and elevated in 47.6 and 39.8% of GC cases respectively with a mean fold change of 0.63, 2.93 and 2.43. The data revealed that reduced mRNA expression of ARID1A and elevated mRNA expression of TP53 and PDL1 was significantly associated with the high-grade and advanced stage of cancer. Our study proposes that ARAD1A under-expression and overexpression of TP53 and PDL1 might be crucial for tumor progression with TP53 and PDL1 acting synergistically.

Keywords: gastric cancer, ARID1A, mutation, mRNA expression 3, mRNA expression, qRT-PCR, TP53, PDL1

INTRODUCTION

Gastric cancer (GC) is prevalent and account for a large number of cancer deaths globally. Although there are considerable advances in cancer diagnosis and therapy, GC remains an important cancer worldwide and is responsible for over one million new cases in 2020 and an estimated 769,000 deaths, ranking fifth for incidence and fourth for mortality globally [1]. In Kashmir valley (North India), GC has been reported as the most frequently diagnosed cancer with an occurrence of around 18.8% among all cancer cases [2].

Multiple genetic and epigenetic alterations in oncogenes and tumor suppressor genes are involved in the process of gastric carcinogenesis [3]. The ARID1A gene encodes a component of the switch/sucrose
non-fermentable (SWI-SNF) chromatin remodelling complex [4].
ARID1A is a helix-turn-helix, nucleoeytoplasmic protein of
approximately 250 kDa, whose stability varies according to its
subcellular location [5]. ARID1A gene expression is down-
regulated in S and G2/M phases and is up-regulated in the G0/G1
phase, which supports the role of ARID1A at the G1 checkpoint for
the proper arrest of cell cycle progression [6]. ARID1A is involved in
the modulation of various cellular processes that are vital in
preventing tumor initiation and progression via regulating the
downstream transcriptional activity of several proto-oncogenes
and tumor suppressor genes (TSGs) [7]. ARID1A gene possesses
a high frequency of somatic mutation in several types of
malignancies leading to reduced or loss of expression, which in
turn exhibits a positive correlation with tumorigenicity [6, 8].
Limited studies have evaluated the possible role of ARID1A so
far. Loss of ARID1A expression was reported in 11–51.3% of
GCs and related to poor clinical parameters and shorter survival
of GC patients [9-10].

TP53 is a well-studied tumor suppressor gene that plays a key role
in regulating the cell cycle. It is a principal mediator of growth arrest,
stenescence and apoptosis in response to a broad array of cellular
damage [11]. Interestingly, The SWI/SNF complex interacts directly
or indirectly with TP53 and regulates the transcription of target
genes downstream of TP53, thereby suggesting that ARID1A plays
important roles in tumor suppression [4, 12]. Some studies have
reported that mutation and aberrant expression of TP53 are
negatively associated with ARID1A loss [13].

PDL1, a molecule able to down-regulate immune response, is
thought to play an important role in the persistence of chronic
infections and evasion of immune destruction by tumor cells [14].
PDL1 acts as a T-cells inhibitor mainly by limiting T-cells activity
within neoplastic tissues and its ligand, PDL1, is often overexpressed
on tumor cells [15]. As per previous studies, PDL1 was
overexpressed in various cancers including GC [16-18]. However,
the prognostic relevance of PDL1 protein expression in GC remains
controversial, and prior studies have shown that PDL1 plays a
promotive or suppressive role in GC [19]. It was also reported
that ARID1A expression is related to PDL1 levels in various cancers
[16-18].

At present, the outcome for patients with advanced GC is still
bleak [20]. Efforts have been directed toward identifying novel
biomarkers for aggressive disease and new molecular targets for
therapeutic intervention. Therefore, in this study, we investigated
the mutation spectrum on exon-9 and expression of ARID1A
along with expression of TP53 and PDL1 genes in GC.
Furthermore, we also correlate the expression with various
clinicopathological parameters to have an idea about the role
of ARID1A in the genesis of GC.

**MATERIALS AND METHODS**

**Study Design**

This was a cross-sectional study conducted by the Department of
Biochemistry and General Surgery, Government Medical College
Srinagar and Associated Shri Maharaja Hari Singh (SMHS) and
Superspeciality Hospital, Srinagar, Kashmir, J&K, India.

**Study Subjects and Sample Collection**

The study included histopathologically confirmed 103 Gastric
tumor tissue samples along with their adjacent normal tissue
collected from the Department of Surgery, Shri Maharaja Hari
Singh (SMHS) Hospital, Srinagar from March 2017 to March 2020. Adjacent normal tissue contained normal gastric mucosa
present near the margins of resection, away from the tumor. The
clinicopathological information of the patients was obtained from
the Medical Records Department of hospital. All the GC cases
were newly diagnosed intestinal type adenocarcinomas and did
not receive any chemo or radiotherapy. The included GC cases
were not having any other type of tumour and were free from any
genetic disorder. One aliquot of tissue sample was snap-frozen
immediately and stored at −80°C till further processing for DNA
analysis. Another aliquot of tissue sample was immediately stored
in RNA-later (Sigma-Aldrich, United States) at 4°C overnight,
to allow the solution to thoroughly penetrate the tissue before stored
at −80°C to prevent any degradation until RNA isolation.

**DNA Isolation**

DNA was extracted from tissue samples using QIAamp DNA
Mini kit (Qiagen, Germany) according to the given protocol. The
quality of the DNA was verified using 1% agarose gel
electrophoresis. The concentration and purity of DNA was measured using NanoDrop 2000c Spectrophotometer
(ThermoFisher Scientific, United States). The DNA samples of
high molecular weight, without any fragmentation/shearing, with
OD (260/280) ratio between 1.8 and 1.9 were processed for
further molecular analysis.

**Polymerase Chain Reaction Followed by**
**DNA Sequencing**

Exon-9 of ARID1A gene was amplified using primers: forward:
5′CACAGCACTATTTGGCTCCAG-3′; reverse: 5′-ATCATC
TCTGGGCTGGCT-3′ (Eurofins Genomics, Germany). The
PCR amplification was carried out in a 50 µl volume containing
12.5 µl of 2X PCR master mix (3B BlackBio, Biotech, India),
0.2 µM of each forward and reverse primers, 50–150 ng of
genomic DNA. After initial denaturation at 94°C for 7 min, 35
cycles of denaturation at 94°C for 20 s, annealing at 58°C for
30 s, and extension at 72°C for 30 s were performed. The final
extension was given at 72°C for 7 min. The 343 bp amplified
product was verified on 2.5% agarose gel and visualized on
Omega Lum G Gel Documentation centre (Aplegen, United
States).

**DNA Sequencing**

The amplified samples were sequenced, using ABI prism 310 automated DNA sequencer (ThermoFisher Scientific, United States) via Sanger dideoxy method.

**RNA Isolation Followed by cDNA Synthesis**

Total RNA was extracted from tissue samples using TRIzol
reagent (ThermoFisher Scientific, United States). The
concentration and purity of RNA was measured using
NanoDrop 2000c Spectrophotometer (ThermoScientific,
RNA having A260/A280 between 1.8 and 2.0 was considered as “Uncontaminated.” Furthermore, RNA integrity was also verified by the presence of 28S, 18S and 5S rRNA bands on 1.5% agarose gel. The first strand cDNA was synthesized with DNase-treated RNA; 1–2 µg RNA was reverse transcribed into cDNA using RevertAid First Strand cDNA Synthesis Kit (ThermoFisher Scientific, United States), according to the manufacturer’s instructions. The reactions were incubated for 60 min at 37°C followed by 95°C for 10 min.

Quantitative Real Time-PCR for Relative mRNA Expression of ARID1A, TP53 and PDL1

qRT-PCR was performed using 7500 Real-Time PCR system (ThermoFisher Scientific, United States). The primers sequences used were; ARID1A forward 5′-CTTCAACCTCAGTCACCTCCA-3′, ARID1A reverse 5′-GGTCAACCCACCTCATACTCTTT-3′; TP53 forward 5′-TGCGGTGGATGTGATTGATGATGG-3′, TP53 reverse 5′-TGGTACAGTGACAGTCAGC-3′; PDL1 forward 5′-AGACAAATTAGTGACGGCCAGCTC-3′, PDL1 reverse 5′-TCCTCCTGTTGGCAGTCAGC-3′; GAPDH forward 5′-CTCCTCTGTTGGCAGTCAGC-3′; GAPDH reverse, 5′-CCCAATCGACCACATCCGG-3′ [10, 21-22]. The GAPDH (housekeeping gene) was used as an internal control. The PCR reaction mixture contained 10 µl of KAPA SYBR® FAST master mix (Sigma-Aldrich, United States), 0.5 µl of cDNA of each sample, 0.2 µM of each forward and reverse primers in a final volume of 20 µl adjusted with Milli-Q water. The reaction mix was preheated at 95°C for 10 min and then amplified with 40 cycles at 95°C for 30 s, X°C for 1 min (X = 60°C for ARID1A; 65°C for TP53, 56°C for PDL1) and 72°C for 35 s. Specificity of the PCR products was determined by Melting curve analysis. All the samples were run in triplicates. The mRNA expression of ARID1A was defined on the basis of C t (cycle threshold) value of each sample. The relative expression was calculated by the 2 -ΔΔC t method [23].

Statistical Analysis

Data analysis was performed using SPSS software V 23.0 (SPSS Inc., Chicago IL, United States). The association between ARID1A, TP53, PDL1 alterations and various sociodemographic and clinicopathological characteristics was evaluated by Pearson’s χ2 test or Fisher’s exact test for discrete variables; paired t-test for continuous variables using multiple logistic regression analysis. The odds ratios (ORs) with 95% confidence intervals (CIs) was calculated. Two-sided p ≤ 0.05 was considered statistically significant.

RESULTS

Patient Characteristics

Out of all GC patients taken for the study 59.3% (61 of 103) were males whereas 40.7% (42 of 103) were females. The mean age (in years) of GC cases was 56.6 ± 12.1. The mean BMI in kg/m² of cases was 24.85 ± 4.45, while as the mean CEA levels of GC cases was 6.07 ± 2.04 ng/ml 57.3% of GC cases were non-smokers and 42.7% were smokers. H. Pylori status was positive in 36.9% (38 of 103) of GC patients. The detailed socio-demographic and clinicopathological parameters of GC patients are given in Table 1.

Mutational Analysis of ARID1A Gene

In the present study, Gastric tumor tissue samples were screened along with their adjacent non-tumor tissue samples for the presence of mutations, if any. On DNA sequencing of amplified exon-9 of ARID1A gene, we detected a nonsense mutation (c. 3219 C > T) at amino acid position 1073, among 02 out of 103 (~2.0%) GC tumor tissues samples leading to formation of stop codon (CAG to TAG). Mutation was not found in any of the adjacent normal tissue samples. Figure 1 shows the partial electrophorograms depicting the c.3219; C > T mutation in exon-9 of ARID1A gene.
Relative mRNA Expression of ARID1A, TP53, PDL1 in GC

We performed qRT-PCR to investigate ARID1A, TP53 and PDL1 mRNA expression in 103 Gastric tumor tissues and their adjacent normal tissue samples. The melt curve analysis showed zero formation of any non-specific products. Figures 2–4 contains box and whisker plots depicting the relative mRNA expression of ARID1A, TP53 and PDL1 in GC cases in terms of their ΔCt values. There was a significant decrease in the mRNA expression of ARID1A in Gastric tumor tissues compared to adjacent normal tissues (ΔCt tumor vs. ΔCt adjacent normal; p < 0.0001; Table 2) with a mean fold change of 0.63. In addition, there was a significant increase in the mRNA expression of TP53 and PDL1 in Gastric tumor tissues compared to adjacent normal tissues (ΔCt tumor vs. ΔCt adjacent normal; p < 0.0001; Table 2) with a mean fold change of 2.93 and 2.43 respectively.

In the present study, “increased” mRNA expression was defined as N-fold ≥2.0, “normal” expression was an N-fold ranging from 0.5001 to 1.9999, and “decreased” mRNA expression was N-fold ≤0.5 [24]. Using this criteria, the relative mRNA expression of the ARID1A was decreased in 25.24% (26 of 103) of GC patients (Table 3). The relative mRNA expression of TP53 and PDL1 was increased in 47.6% (49 of 103) and 39.8% (41 of 103) of GC patients respectively (Tables 4, 5).

Association of Relative mRNA Expression With Various Parameters of GC Cases

The stratification of ARID1A mRNA levels with respect to various socio-demographic and clinicopathological parameters of GC patients is given in Table 3. Among GC cases having stage I&II disease only 18.6% were having reduced ARID1A mRNA expression compared to 39.4% patients with stage III&IV disease and reduced ARID1A mRNA expression (OR = 2.8; p = 0.03). A higher percentage (37.9%) of GC patients with poorly differentiated disease were having reduced ARID1A mRNA expression compared to GC patients with well differentiated disease (18.2%) (OR = 2.7; p = 0.03).

The stratification of TP53 mRNA levels with respect to various socio-demographic and clinicopathological parameters of GC patients is given in Table 4. A significantly higher percentage of Preobese GC patients were having elevated TP53 mRNA levels compared to GC patients having normal BMI and elevated TP53 mRNA levels (67.9 vs. 31.5%; OR = 6.3; p = 0.0002). Among GC cases without H. Pylori infection 35.4% (23/65) were having TP53 mRNA overexpression compared to GC patients with H. Pylori infection among which 68.4% (26/38) were having TP53 overexpression (OR = 4.0; p = 0.002). Among GC cases having stage I & II disease only 38.0% were having reduced TP53 mRNA expression compared to 68.8% patients with stage III&IV disease and elevated TP53 mRNA expression (OR = 3.6; p = 0.005). A higher percentage of GC patients with poorly differentiated disease were having elevated TP53 mRNA expression compared to GC patients with well differentiated disease (34.4 vs. 69.2%; OR = 4.3; p = 0.001).

The stratification of PDL1 mRNA levels with respect to various socio-demographic and clinicopathological parameters of GC patients is given in Table 5. A significantly higher percentage of GC patients with stage III & IV disease were having elevated PDL1 mRNA levels compared to GC patients having stage I & II disease (71.9 vs. 25.4%; OR = 7.6; p = 0.00). Among GC cases with well differentiated disease only 21.9% (14/66) were having elevated PDL1 mRNA expression compared to GC patients having stage I & II disease (25.4%; OR = 3.6; p = 0.005). A higher percentage of GC patients with poorly differentiated disease were having elevated PDL1 mRNA overexpression compared to GC patients with well differentiated disease among which 69.2% (27/37) were having PDL1 overexpression (OR = 8.1; p = 0.00).

Relationship Between ARID1A, TP53 and PDL1 mRNA Expression

Figure 5 represents a venn diagram depicting the correlation between ARID1A mRNA underexpression and mRNA overexpression of TP53 and PDL1. Only TP53 overexpression and PDL1 overexpression were significantly associated with each other and the correlation was positive (OR = 7.6; p = 0.00; Table 4).

DISCUSSION

The clinical symptoms of GC are often commenced at an advanced stage, resulting in the limitation of diagnosis and therapeutic approaches to around 50% of cancerous cases [25].
However, over the past decade, there has been a striking improvement in cancer management and treatment by understanding the genetics of disease.

ARID1A has recently emerged as a novel tumor suppressor gene, as per the evidence supporting the positive association between reduced ARID1A expression and tumorigenicity of...
several cancers, such as ovarian, endometrial, cervical, breast, gastric, colorectal, and lung cancers [7, 10, 26]. We investigated the status of ARID1A mutation in GC wherein we detected a nonsense mutation (c.3219; C > T) among two (∼2.0%) GC patients that introduces a premature stop codon leading to the abortive termination of the ARID1A protein, thereby resulting in its complete or partial inactivation and reduced or loss of protein function. Mamo et al. previously reported nonsense mutation in exon-9, which introduces the premature stop codon into coding sequence at position W1073 [27]. Furthermore, studies have reported that frequency of this nonsense mutation is usually low in tumor cells [27, 28]. However, the general mutation rate of the ARID1A gene varies between 8 and 39% in gastrointestinal cancers [29] and between 8 and 29% in GC [26]. Earlier studies have demonstrated that ARID1A mutations were more frequent in Gastric tumors, especially with microsatellite instability and Epstein-Barr virus infection [10, 29]. The nonsense mutation of ARID1A, resulting in loss of its functional protein, consequently leads to the activation of the PI3K/AKT pathway that promotes several mechanisms responsible for carcinogenesis, including cell proliferation, inhibition of apoptosis, cell adhesion, and transformation [5, 30]. Several studies have reported that the siRNA knockdown of ARID1A increases phosphorylations of AKT and promotes cell division and metastasis [5, 30].

Our result revealed that ARID1A was under-expressed at mRNA level in 25.6% of GC cases with an average fold change of 0.63. Consistent with our study, Yang et al. found that the expressional loss of ARID1A was frequent in 30% of GC patients and has a significant correlation with poor survival and prognosis [31]. However, Wang et al. revealed that ARID1A was significantly lower in 65.15 and 52% of GC patients at mRNA and protein level respectively [10]. Previously it has been reported that about 30% of Caucasians, 25% of Asians and 10% of Pacific Islanders exhibited loss of ARID1A expression in clear cell and endometrioid ovarian carcinoma [31]. Additionally, there was no significant difference when comparing individuals of Japanese and non-Japanese origin with respect to their decreased ARID1A expression in ovarian cancer (29 vs. 18%) [31]. The expressional loss of ARID1A has been shown to trigger the initiation and progression of carcinogenesis in numerous types of cancers, including ovarian, breast, endometrial and cervical, breast, gastric, and colorectal cancers through several mechanisms.

![Box and whisker plot depicting the relative mRNA expression of PDL1 in terms of ΔCt values of GC tumor tissues and adjacent normal tissues (controls). The experiment was performed in triplets. The relative mRNA expression of PDL1 was significantly lower in Gastric tumors tissue samples compared to adjacent non-tumorous tissues (p < 0.001). Data was represented as mean ± SD.](image)
that have not been fully elucidated [26]. Studies have reported that restoration of normal ARID1A protein levels in vitro successfully inhibits the uncontrolled cell division [26]. Chan-on et al. observed that knockdown of ARID1A in three wild-type cell lines promotes the cell division in bile duct cancer, and the effect was reversed when ARID1A was ectopically re-expressed [32]. We further investigated the association of GC with clinicopathological parameters and found lower ARID1A expression in patients with the high-grade and advanced stage of the GC. Consistent with our findings, some investigations also reported that the reduced expression of the ARID1A has a significant association with higher grading and staging of GC [10]. However, several studies have also reported that loss of ARID1A expression has no significant association with advanced cancer grade and stage [33]. Although not found in our study, Chou et al. revealed that ARID1A has a significant association with age, gender, tumor location, and tumor size [34]. Our study confirmed that the mRNA levels of ARID1A were low in the two GC patients with ARID1A c.3219 C > T mutation. Consistent with our study, Mamo et al. also reported that RNA levels of ARID1A were found very low in samples with nonsense mutations [27]. The introduction of stop codon in upstream region results in abortive termination of proteins that could interfere the normal protein functions [35].

The role of TP53 in suppressing tumour growth is primarily due to its induction of cell cycle arrest and DNA repair or apoptosis, following genotoxic stress [36]. We observed a significantly higher TP53 relative mRNA expression in 47.6% TABLE 3 | Association of ARID1A mRNA expression with socio-demographic and clinicopathological variables of GC patients.

| Variables                  | Cases N = 103 (%) | ARID1A mRNA expression | OR (95% CI) | p-Value |
|----------------------------|-------------------|------------------------|-------------|---------|
|                            |                   | Normal 77 (74.8%)      | Reduced 26 (25.2%) |         |
|                            |                   | 42 (68.8)              | 19 (31.2)    | 1.00    | 0.10   |
|                            |                   | 35 (83.3)              | 07 (16.7)    | 0.4 (0.15–1.6) |
| Gender                     | Male              | 61 (59.)               | 42 (68.8)    | 19 (31.2) | 1.00 |
|                            | Female            | 42 (40.7)              | 35 (83.3)    | 07 (16.7) | 0.4 |
| Age group                  | <50 years         | 35 (34.0)              | 28 (80.0)    | 07 (20.0) | 1.00 |
|                            | ≥50 years         | 68 (66.0)              | 49 (72.0)    | 19 (28.0) | 1.5 (0.6–4.4) |
| Dwelling                   | Rural             | 66 (64.0)              | 51 (77.2)    | 15 (22.8) | 1.00 |
|                            | Urban             | 37 (36.0)              | 26 (70.2)    | 11 (29.8) | 1.4 (0.56–3.6) |
| Smoking status             | Non-Smoker        | 59 (57.3)              | 47 (80.0)    | 12 (20.0) | 1.00 |
|                            | Smoker            | 44 (42.7)              | 30 (68.1)    | 14 (31.9) | 1.8 (0.73–4.5) |
| BMI (kg/m²)                | Normal            | 54 (52.4)              | 41 (76.0)    | 13 (24.0) | 1.00 |
|                            | Underweight       | 10 (9.7)               | 06 (60.0)    | 04 (40.0) | 2.1 (0.45–8.8) |
|                            | Preobese          | 28 (27.2)              | 22 (78.5)    | 06 (21.5) | 0.9 (0.27–2.8) |
|                            | Obese Class I     | 09 (8.7)               | 00 (33.3)    | 09 (66.7) | 1.6 (0.3–7.2) |
|                            | Obese Class II    | 02 (1.9)               | 02 (100.0)   | 00 (0.0)  | 1.0 (0.03–10.2) |
| Family history             | No                | 86 (83.5)              | 65 (75.5)    | 21 (24.5) | 1.00 |
|                            | Yes               | 17 (16.5)              | 12 (70.5)    | 05 (29.5) | 1.3 (0.37–4.0) |
| Salt tea consumption       | <5 cups/day       | 29 (28.2)              | 19 (65.5)    | 10 (34.5) | 1.00 |
|                            | ≥5 Cups/day       | 74 (71.8)              | 58 (78.3)    | 16 (21.7) | 0.53 (0.2–1.4) |
| CEA levels (ng/ml)         | Normal            | 34 (33.0)              | 23 (67.6)    | 11 (32.4) | 1.00 |
|                            | Elevated          | 69 (67.0)              | 54 (78.2)    | 15 (21.8) | 0.58 (0.2–1.4) |
| H. Pylori                  | Absent            | 65 (63.1)              | 51 (78.4)    | 14 (21.6) | 1.00 |
|                            | Present           | 38 (36.9)              | 26 (68.4)    | 12 (31.6) | 1.7 (0.66–4.2) |
| Stage                      | I and II          | 70 (68.0)              | 57 (81.4)    | 13 (18.6) | 1.00 |
|                            | III and IV        | 33 (32.0)              | 20 (60.6)    | 13 (39.4) | 2.8 (1.1–7.2) |
| Grade                      | WD                | 66 (64.0)              | 54 (81.8)    | 12 (18.2) | 1.00 |
|                            | PD                | 37 (36.0)              | 23 (62.1)    | 14 (37.9) | 2.7 (1.1–6.9) |
| TP53 mRNA expression       | Normal            | 54 (52.4)              | 39 (72.2)    | 15 (27.8) | 1.00 (Ref) |
|                            | Reduced           | 49 (47.6)              | 38 (72.2)    | 11 (27.8) | 0.7 (0.3–1.8) |
| PDL1 mRNA expression       | Normal            | 62 (60.2)              | 46 (74.1)    | 16 (25.8) | 1.00 (Ref) |
|                            | Elevated          | 41 (39.8)              | 31 (75.6)    | 10 (24.4) | 0.9 (0.3–2.3) |

BMI, basal metabolic index (<18.5 = underweight, 18.5–24.99 = Normal, 25–29.99 = Preobese, 30–34.99 = Obese class I, 35–39.99 = Obese class II).

CEA, carcinoembryonic antigen; H. Pylori, Helicobacter pylori; WD, well differentiated; PD, poorly differentiated.
of GC cases. The mean level of TP53 mRNA expression was almost 3 fold higher in GC tumours than in adjacent normal mucosa. In our study, some GC tumours showed high TP53 mRNA levels, while others showed a slight increase. So, TP53 mRNA may either be weakly expressed in all tumour cells or highly expressed in a few tumour cells owing to tumour heterogeneity. Our results clearly demonstrate that TP53 regulation may occur at a pretranslational step, involving either an increase in TP53 gene expression and/or stabilization of its mRNA which might lead to elevated content of TP53 protein in the cell. Although there is a particular paucity of studies that have analysed the relative expression of TP53 mRNA in cancer, TP53 has been shown to express at a high frequency in gastric adenocarcinomas [37]. In line with our observation, TP53 mRNA expression was significantly higher in triple-negative breast cancer (TNBC) [38]. Our findings confirm earlier reports, which showed an elevated level of TP53 transcripts in 70% [39] and 66% [40] of tested CRC tumours respectively. Overexpression of TP53 mRNA has recently been shown to increase the amount of endogenous TP53 and to increase apoptosis in human melanoma cells, in part, by modulating the transcription of downstream target genes including downregulation of p21 and upregulation of TP53-induced death domain protein, to favour apoptosis rather than cell cycle arrest [41]. Reports suggest that overexpressed mRNA can enhance or inhibit the ability of TP53 to trans activate certain target promoters and to induce apoptosis [42]. Thus, regulated expression of TP53 isoforms is critical for the biological outcome of TP53.

On stratification, we observed a significant association of elevated TP53 mRNA levels with higher stage and higher grade of GC. In tune with our observations, Fenoglio-Preiser

### Table 4

| Variables                        | Cases N = 103 (%) | TP53 mRNA expression | OR (95% CI) | p-Value |
|----------------------------------|-------------------|-----------------------|-------------|---------|
|                                  |                   | Normal 54 (52.4)   | Elevated 49 (47.6) |         |
| Gender                           |                   |                      |             |         |
| Male                             | 61 (59.3)         | 29 (47.5)            | 32 (52.5)   | 1.00 (Ref.) | 0.3   |
| Female                           | 42 (40.7)         | 25 (59.5)            | 17 (40.5)   | 0.6 (0.3-1.4) |       |
| Age group                        |                   |                      |             |         |
| <50 years                        | 35 (34.0)         | 21 (60.0)            | 14 (40.0)   | 1.00 (Ref.) | 0.3   |
| ≥50 years                        | 68 (66.0)         | 33 (48.5)            | 35 (51.5)   | 1.6 (0.7-3.6) |       |
| Dwelling                         |                   |                      |             |         |
| Rural                            | 66 (64.0)         | 37 (56.1)            | 29 (43.9)   | 1.00 (Ref.) | 0.4   |
| Urban                            | 37 (36.0)         | 17 (45.9)            | 20 (54.1)   | 1.5 (0.7-3.3) |       |
| Smoking status                   |                   |                      |             |         |
| Non-Smoker                       | 59 (57.3)         | 36 (59.0)            | 25 (41.0)   | 1.00 (Ref.) | 0.1   |
| Smoker                           | 44 (42.7)         | 18 (42.9)            | 24 (57.1)   | 1.9 (0.8-4.2) |       |
| BMI (kg/m²)                      |                   |                      |             |         |
| Normal                           | 54 (52.4)         | 37 (68.5)            | 17 (31.5)   | 1.00 (Ref.) |       |
| Underweight                      | 10 (9.7)          | 05 (50.0)            | 05 (50.0)   | 2.1 (0.5-9.0) | 0.2   |
| Preobese                         | 26 (27.2)         | 07 (52.1)            | 21 (47.9)   | 6.3 (2.3-18.9) | 0.0002 |
| Obese Class I                    | 09 (9.7)          | 04 (44.4)            | 05 (55.6)   | 2.6 (0.6-12.5) | 0.2   |
| Obese Class II                   | 02 (1.9)          | 01 (100.0)           | 01 (0.0)    | 2.1 (0.05-8.7) | 0.6   |
| Family history                   |                   |                      |             |         |
| No                               | 86 (83.5)         | 47 (53.4)            | 41 (46.6)   | 1.00 (Ref.) | 0.7   |
| Yes                              | 17 (16.5)         | 07 (46.7)            | 08 (53.3)   | 1.3 (0.4-3.9) |       |
| Salt tea consumption             |                   |                      |             |         |
| <5 cups/day                      | 29 (28.2)         | 19 (65.5)            | 10 (34.5)   | 1.00 (Ref.) | 0.1   |
| ≥5 Cups/day                      | 74 (71.8)         | 35 (47.3)            | 39 (52.7)   | 2.1 (0.8-5.3) |       |
| CEA levels (ng/ml)               |                   |                      |             |         |
| Normal                           | 34 (33.0)         | 37 (53.6)            | 32 (46.4)   | 1.00 (Ref.) | 0.8   |
| Elevated                         | 69 (67.0)         | 17 (50.0)            | 17 (50.0)   | 1.2 (0.5-2.6) |       |
| H. Pylori                        |                   |                      |             |         |
| Absent                           | 65 (63.1)         | 42 (64.6)            | 23 (35.4)   | 1.00 (Ref.) | 0.8   |
| Present                          | 38 (36.9)         | 12 (31.6)            | 26 (68.4)   | 4.0 (1.7-9.2) |       |
| Stage                            |                   |                      |             |         |
| I and II                         | 70 (68.0)         | 44 (62.0)            | 27 (38.0)   | 1.00 (Ref.) | 0.005 |
| III and IV                       | 33 (32.0)         | 10 (31.3)            | 22 (68.8)   | 3.6 (1.5-8.7) |       |
| Grade                            |                   |                      |             |         |
| WD                               | 66 (64.0)         | 42 (65.6)            | 24 (34.4)   | 1.00 (Ref.) | 0.001 |
| PD                               | 37 (36.0)         | 12 (30.8)            | 27 (69.2)   | 4.3 (1.3-10.0) |       |
| PDL1 mRNA expression             |                   |                      |             |         |
| Normal                           | 62 (60.2)         | 44 (71.0)            | 18 (29.0)   | 1.00 (Ref.) | 0.00  |
| Elevated                         | 41 (39.8)         | 10 (24.4)            | 31 (75.6)   | 7.6 (3.1-18.6) |       |

BMI, basal metabolic index (<18.5 = underweight, 18.5–24.99 = Normal, 25–29.99 = Preobese, 30–34.99 = Obese class I, 35–39.99 = Obese class II). CEA, carcinoembryonic antigen; H. Pylori, Helicobacter pylori; WD, well differentiated; PD, poorly differentiated.
et al. has reported TP53 overexpression in almost 90% of invasive Gastric tumors [43]. Increased expression of TP53 mRNA in ovarian and renal cell carcinoma has been associated with worse prognosis and higher tumour grade [44]. As per previous studies, the degree of TP53 expression correlates positively with the proliferative rate of the tumors [45] and there is a tendency for TP53 expression to be more common in poorly differentiated tumors than in well differentiated lesions [46]. Kakeji et al. [47] showed that tumors with TP53-positive staining had a higher proliferative activity than did those that stained negative. Previously, breast cancer tumors of highly malignant potential and poor prognosis showed higher expression of TP53 protein [21]. In contradiction with our study, TP53 mRNA overexpression was associated with lower recurrence rates and higher overall survival rates in breast cancer and gastric cancer [37, 48]. In addition, no correlation was found between TP53 mRNA, tumour stage and disease prognosis in CRC and GC [49, 50]. Similarly, as per few previous studies, no correlation was found between positive TP53 tissue status and histological grade of tumor differentiation [51]. It has been suggested that TP53 mRNA also harbours information that helps control TP53 protein turnover rate [52].

In our study, most of the GC patients with H. Pylori infection had significantly increased TP53 mRNA levels. Shiao et al. observed overexpression of TP53 in 15% of H. Pylori–positive chronic gastritis patients but 38% of H. Pylori–positive metaplastic gastritis patients [53]. It has been confirmed that infection from H. Pylori is a major cause of chronic inflammation of the human gastric antral mucosa leading to development of atrophic chronic gastritis and Gastric carcinoma (CG) [54]. In consonance with our study, it has been shown that H. Pylori infection increased TP53 expression and the apoptosis rate in GC [55]. Wei et al. found that TP53 levels before H. Pylori infection were low or undetectable and were elevated on exposure to H. Pylori infection accompanied with intense inflammation [56]. According to a study by Ahmed et al. cells cultivated with H. Pylori were found to be in phase G1 of the cell cycle with TP53 overexpression suggesting that cell cycle arrest in G1 is associated

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**TABLE 5** | Association of PDL1 mRNA expression with socio-demographic and clinicopathological variables of GC patients.

| Variables          | Cases N = 103 (%) | PDL1 mRNA expression | OR (95% CI) | p-Value |
|--------------------|------------------|----------------------|-------------|--------|
|                    |                  | Normal 62 (60.2)     | Elevated 41 (39.8) |        |
| Gender             |                  |                      |             |        |
| Male               | 61 (59.3)        | 36 (59.0)            | 25 (41.0)   | 1.00 (Ref.) | 0.8 |
| Female             | 42 (40.7)        | 26 (61.9)            | 16 (38.9)   | 0.8 (0.4–2.0) | 0.8 |
| Age group          |                  |                      |             |        |
| <50 years          | 35 (34.0)        | 22 (62.9)            | 13 (37.1)   | 1.00 (Ref.) | 0.8 |
| ≥50 years          | 68 (66.0)        | 40 (58.8)            | 28 (41.2)   | 1.2 (0.5–2.7) | 0.8 |
| Dwelling           |                  |                      |             |        |
| Rural              | 66 (64.0)        | 42 (63.6)            | 24 (36.4)   | 1.00 (Ref.) | 0.4 |
| Urban              | 37 (36.0)        | 20 (54.1)            | 17 (45.9)   | 1.5 (0.6–3.4) | 0.4 |
| Smoking status     |                  |                      |             |        |
| Non-Smoker         | 59 (57.3)        | 45 (73.8)            | 16 (26.2)   | 1.00 (Ref.) | 0.001 |
| Smoker             | 44 (42.7)        | 17 (40.5)            | 25 (59.5)   | 4.1 (1.8–9.6) | 0.001 |
| BMI (kg/m²)        |                  |                      |             |        |
| Normal             | 54 (52.4)        | 36 (66.7)            | 18 (33.3)   | 1.00 (Ref.) | 0.1 |
| Underweight        | 10 (9.7)         | 09 (80.0)            | 01 (20.0)   | 0.2 (0.009–1.5) | 0.08 |
| Preobese           | 28 (27.2)        | 13 (46.4)            | 15 (53.6)   | 2.2 (0.9–5.9) | 0.07 |
| Obese Class I      | 09 (8.7)         | 03 (33.3)            | 06 (66.7)   | 3.9 (0.8–21.1) | 0.07 |
| Obese Class II     | 02 (1.9)         | 01 (50.0)            | 01 (50.0)   | 1.9 (0.4–8.1) | 0.6 |
| Family history     |                  |                      |             |        |
| No                 | 86 (83.5)        | 51 (68.0)            | 37 (32.0)   | 1.00 (Ref.) | 0.4 |
| Yes                | 17 (16.5)        | 11 (73.3)            | 04 (26.7)   | 0.5 (0.1–1.7) | 0.4 |
| Salt tea consumption |          |                      |             |        |
| <5 cups/day        | 29 (28.2)        | 20 (70.0)            | 09 (30.0)   | 1.00 (Ref.) | 0.2 |
| ≥5 cups/day        | 74 (71.8)        | 42 (56.7)            | 32 (43.3)   | 1.68 (0.7–4.3) | 0.9 |
| CEA levels (ng/ml) |                  |                      |             |        |
| Normal             | 34 (33.0)        | 42 (60.9)            | 27 (39.1)   | 1.00 (Ref.) | 0.9 |
| Elevated           | 69 (67.0)        | 20 (66.8)            | 14 (33.3)   | 1.1 (0.5–2.5) | 0.06 |
| H. Pylori           |                  |                      |             |        |
| Absent             | 65 (63.1)        | 44 (67.7)            | 21 (32.3)   | 1.00 (Ref.) | 0.00 |
| Present            | 38 (36.9)        | 18 (47.4)            | 20 (52.6)   | 2.3 (1.1–5.3) | 0.00 |
| Stage              |                  |                      |             |        |
| I and II           | 70 (68.0)        | 53 (74.6)            | 18 (25.4)   | 1.00 (Ref.) | 0.00 |
| Ill and IV         | 33 (32.0)        | 09 (28.1)            | 23 (71.9)   | 7.5 (2.9–19.2) | 0.00 |
| Grade              |                  |                      |             |        |
| WD                 | 66 (64.0)        | 50 (78.1)            | 14 (21.9)   | 1.00 (Ref.) | 0.00 |
| PD                 | 37 (36.0)        | 12 (30.8)            | 25 (69.2)   | 8.1 (3.3–19.8) | 0.00 |

BMI, basal metabolic index (<18.5 = underweight, 18.5–24.99 = Normal, 25–29.99 = Preobese, 30–34.99 = Obese class l, 35–39.99 = Obese class II).

CEA, carcinoembryonic antigen; H. pylori, Helicobacter pylori; WD, well differentiated; PD, poorly differentiated.
with a reduction in cyclin E levels and an increase in TP53 and p21 expression showing that H. Pylori can induce cell stress, reduce the ability to repair damaged cells, and can increase the number of changes in the genome, leading to genetic instability and finally to GC [57].

PD1 acts as a T-cells inhibitor mainly by limiting T-cells activity within neoplastic tissues and its ligand, PDL1, is often overexpressed on tumor cells [18]. In our study, PDL1 relative mRNA expression was significantly higher in 39.8% of GC cases with an average fold change of 2.43 in tumor tissues compared to adjacent normal tissues. Wu et al. showed that immunohistochemical PDL1 expression was strongly positive in 42.2% of 102 human gastric carcinomas, weakly positive in adenoma samples and totally negative in normal gastric tissue [58]. PDL1 expression has been reported in a wide variety of solid tumors, including lung cancer, hepatocellular carcinoma and intra-hepatic cholangiocarcinoma, gastric, colorectal, pancreatic, ovarian, breast, cervical and oral cancer, head and neck squamous cell carcinomas, nasopharyngeal, esophageal, urothelial and renal cell cancer, nephroblastoma, melanoma and gliomas [58, 59]. It has been suggested that CD8+ T cells upregulate PD1 expression and secrete IFN-γ when they encounter tumor antigens, resulting in the upregulation of PDL1 expression on tumor cells and immune cells and the ligation of PDL1 with PD1 will decrease T cell function and create a negative feedback mechanism that decreases antitumor immunity leading to tumorigenesis [22]. In contradiction with our observation, no statistically significant differences were found with regard to PDL1 mRNA levels within normal and GC specimens as previously verified by Chen et al. [60].

On stratification, we observed a significant association of elevated PDL1 mRNA levels with higher stage and higher grade of GC. PDL1 overexpression has been associated to higher number of lymph node metastasis, larger tumor size, increased depth of invasion and poorer overall survival in various cancers [58, 61]. According to a previous study, PD1, PDL1 and CD8 mRNA levels were significantly higher in undifferentiated GC [22]. More recently, it was demonstrated that PDL1 overexpression was a worse prognostic factor in GC [19].

In our study, we observed a positive correlation between TP53 and PDL1 mRNA expression, suggesting that there is a synergistic effect between PDL1 and TP53 in the occurrence and development of tumors, which has also been demonstrated in NSCLC wherein TP53 has been shown to regulate PDL1 expression via miR-34 that binds PDL1 3′-untranslated region in NSCLC models [62, 63]. Moreover, the expression of PDL1 and TP53 has previously been positively correlated [64]. All these studies including ours’ linked tumor immune evasion to other tumor suppressor pathways previously described for TP53 [65].

**Conclusion**

In summary, we observed down regulation of ARID1A mRNA expression and upregulation of TP53 and PDL1 mRNA expression in GC which was in turn significantly associated high-grade and advanced stage of tumor suggesting that lower ARID1A expression and higher expression of TP53 and PDL1 might play a definite role in the initiation and progression of GC. Furthermore, a positive correlation was found between TP53 and PDL1 mRNA expression. This should be useful for future antitumour research and for the design of therapeutic agents specific to the inactivation process. However, further largescale and comprehensive researches are needed to support our results and conclusion.
DATA AVAILABILITY STATEMENT
The data will be made available upon reasonable request.

ETHICS STATEMENT
The study was approved by the Institutional Ethical Committee, Government Medical College Srinagar, Kashmir (No. 66/ETH/GMC). All the samples were collected after taking written informed consent from the patients and proper ethical procedures were followed.

AUTHOR CONTRIBUTIONS
Conceptualization: JQ and MK; Data curation: JQ and MK; Formal analysis: MK; Funding acquisition: SM; Investigation: JQ, MK, MW, FR, and SB; Writing—original draft: JQ and MK; Writing-review and editing: MK; Approval of final manuscript: all authors.

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CONFLICT OF INTEREST
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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