Oncogenic and tumor suppressor genes expression in myeloproliferative neoplasms: The hidden side of a complex pathology

Elham Abedi1 | Mehran Karimi1 | Ramin Yaghobi2 | Hamid Mohammadi3 | Sezaneh Haghpanah1 | Mohamad Moghadam1 | Elahe Bayat4 | Alireza Rezvani1 | Mani Ramzi1

1Hematology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
2Transplant Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
3Department of Pediatrics, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
4Department of Biochemistry, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Correspondence
Mani Ramzi, Hematology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.
Email: ramzim43@yahoo.com

Funding information
This article was funded by Shiraz University of Medical Sciences as a grant for a PhD dissertation (research ID: 97-01-104-17048, grant no. 97-01-32-17090). The funder had no impact on the study design, data analysis, or results interpretation.

Abstract
Background: The present study aimed to explore the changes in the expressions of six tumor-related genes in myeloproliferative neoplasms (MPNs). The study population included 130 patients with MPNs (52 with chronic myeloid leukemia (CML), 49 with essential thrombocythemia (ET), 20 with polycythemia vera (PV), and 9 with primary myelofibrosis (PMF)) and 51 healthy individuals.

Methods: The expression profiling of six genes (ADAMTS18, CMTM5, CDKN2B, DCC, FHIT, and WNT5B) in the peripheral blood granulocyte cells was explored by real-time quantitative reverse transcription polymerase chain reaction.

Results: The patients with MPNs showed significant downregulation of CMTM5 (EFC = 0.66) and DCC (EFC = 0.65) genes in contrast to a non-significant upregulation of ADAMTS18, CDKN2B, FHIT, and WNT5B genes. Downregulation of DCC was consistent in all subtypes of MPN (EFC range: 0.591–0.860). However, CMTM5 had a 1.22-fold upregulation in PMF in contrast to downregulation in other MPN subtypes (EFC range: 0.599–0.775). The results revealed a significant downregulation in CMTM5 and DCC at below 60-years of age. Furthermore, female patients showed a clear-cut downregulation in both CMTM5 and DCC (EFC DCC: 0.436 and CMTM5: 0.570), while male patients presented a less prominent downregulation with a borderline p-value only in DCC (EFC: 0.69; p = 0.05).

Conclusions: Chronic myeloid leukemia cases showed a significant upregulation of WNT5B, as a known oncogenesis gene. Two tumor suppressor genes, namely DCC and CMTM5, were downregulated in the patients with MPNs, especially in females and patients below 60 years of age.

Abbreviations: CALR, calreticulin; CML, chronic myeloid leukemia; ET, essential thrombocythemia; JAK2, Janus kinase; MPN-, BCR-ABL1-negative; MPN+, BCR-ABL1-positive; MPNs, myeloproliferative neoplasms; PMF, primary myelofibrosis; PV, polycythemia vera.
1 | INTRODUCTION

Myeloproliferative neoplasms (MPNs) refer to a heterogeneous group of hematologic disorders. According to the World Health Organization (WHO) (2016), these neoplasms have been divided into three main subgroups based on JAK2/CALR/MPL mutation; that is, Essential thrombocythemia (ET), polycythemia vera (PV), and primary myelofibrosis (PMF), and four clinicopathologic conditions including chronic myeloid leukemia (CML), chronic neutrophilic leukemia (CNL), chronic eosinophilic leukemia, and unclassifiable (MPN-U).1–6 ET, PV, and PMF are BCR-ABL1-negative MPNs, while chronic myeloid leukemia is a BCR-ABL1-positive MPN.6,7 ET and PV are most often present with a rise in the platelet count and hemoglobin/hematocrit, respectively, both of which being accompanied by the risk of hemorrhage and vascular thrombosis. PMF is an advanced subtype of MPNs whose clinical presentations are remarkably more heterogeneous compared to ET and PV. PMF is associated with the release of fibrosis and pro-inflammatory cytokines, bone marrow fibrosis, and often extensive extramedullary hematopoiesis in the spleen or liver. CML is caused by the acquisition of BCR-ABL1 in hematopoietic stem cells, which transforms them into leukemic stem cells (LSC). BCR-ABL1 mutation results from a reciprocal translocation between the long arms of chromosomes 9 and 22, leading chromosome 22 to become shorter.8,9 Molecular monitoring of BCR-ABL1 for CML using the international scale (IS) has become the model for molecular monitoring of other types of leukemia and diseases.10,11

Since the discovery of Jak2V617F followed by CALR and other mutations in patients with MPNs, a great number of genomic studies have revealed more somatic alterations in the majority of these patients. Although the mutational events involved in MPNs pathogenesis have been comprehensively determined, the impact of different somatic alterations on gene expression and transcriptional output has not been evaluated yet. In the MPNs family, especially in CML, some downstream signaling pathways account for the progression of the disease. This factor alongside molecular events alters the expression profiles of several important genes that may play a crucial role in the evolution and pathogenesis of MPNs. Therefore, the present study aimed to assess the expression levels of a number of genes associated with DNA methylation, as the common epigenetic change in hematological cancers. For this purpose, the raw data from GeoDataSet NCBI (GSE87806) and the previous studies were reviewed to identify the most likely genes involved in this process. Accordingly, ADAMTS18, CMTM5, CDKN2B, FHIT, WNT5B, and DCC were among the genes more commonly affected by methylation changes in MPNs.12–19

2 | MATERIALS AND METHODS

In this cross-sectional study, patients with MPNs referred to the hematology-oncology department of Namazee Hospital, Shiraz, Iran from May 2018 to May 2019 were selected as the patient group. A group of age- and sex-matched volunteers was also selected as the control group. The patients were diagnosed based on the WHO’s criteria and clinical, laboratory, and molecular analyses. Written informed consent was obtained from all the participants. The study design was approved by the Ethics Committee of Shiraz University of Medical Sciences, Shiraz, Iran (ethics code: IR.SUMS.REC1397.535).

### Table 1 Primer sequences and PCR product sizes of various genes studied

| Gene   | Primer sequence                          | Amplifier size | Accession No. |
|--------|------------------------------------------|----------------|---------------|
| WNT5B  | F:GCAGCAGCCGGGACAACG<br>R:CGTGGGTGAACGGGCTTC | 75             | NM_032642.3   |
| ADAMTS18 | F:AGCCCAAGCAAGCGCAAGAGTA<br>R:GGGGCAAACCTTGTCCTCA | 190            | NM_199355.4   |
| CMTM5  | F:AAACCGAGCTGGCCCTGAC<br>R:AAAGGAAAGCCAGTGTGTGAA | 109            | NM_001288746.2 |
| CDKN2B | F:CGGGAGTTATGATGATGG<br>R:GGTGGGTAGAGTGGCA | 97             | NM_004936.4   |
| DCC    | F:AGCCAGCAGAGAAAGAAAC<br>R:GGTGGGTAGAGTGGCA | 186            | NM_005215.4   |
| HPRT1  | F:GGGTCGTTATTAGTGATGATGA<br>R:ACCCCTTCCATCTCGACAT | 86             | NM_000194.3   |
| FHIT   | F:GCAAATACCTGCGCTGCTTGA<br>R:ACAAGAGGCGAAGAGACAGT | 179            | NM_002012.4   |
Peripheral blood granulocyte cells were collected from the patient and control groups by density gradient methods using lymphodex (Inno-train). Total RNA was extracted using RiboExTM (GeneAll) according to the manufacturer’s instructions. The quantity and quality of the extracted RNA were measured using the NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific Inc). RNA integrity was also evaluated using electrophoresis on 1% agarose gel. Besides, the synthesis of complementary DNA (cDNA) was done using Prime RT Premix cDNA synthesis kit (Genet Bio) according to the manufacturer’s instructions. The samples were stored at −70°C.

2.2 | ADAMTS18, CMTM5, CDKN2B, DCC, FHIT, and WNT5B mRNA expression

Specific primers for HPRT1 (as the internal control or housekeeping gene) and ADAMTS18, CMTM5, CDKN2B, DCC, FHIT, and WNT5B genes were designed using the AlleleID software (PREMIER). To prevent the genomic DNA amplification exon/exon junction spanning primers or Intron interval primers were designed. The details regarding the quantitative reverse transcription polymerase chain reaction (qRT-PCR) primers have been given in Table 1. The primers were blasted with the entire human genome. The primers used for RT-PCR for all gene amplifications were synthesized by Metabion, Germany (Table 1).

2.3 | Real-time quantitative reverse transcription-polymerase chain reaction

The relative expression profiles of ADAMTS18, CMTM5, CDKN2B, DCC, HPRT1, FHIT, and WNT5B genes were assessed by SYBR Green I real-time PCR Chemistry, with the HPRT1 gene being used as the endogenous control gene. Briefly, PCR reactions were performed in a final volume of 10 μl containing 1 μl of the cDNA, 5 μl of 2X Bio fact SYBR Master Mix (Daejeon), and 0.5 pM of the primer pairs. After initial denaturation at 95°C for 3 min, 40 cycles consisting of the following steps were performed using IQ5 Real-Time PCR System (BIO-RAD the US): 30 seconds at 95°C, 30 s at 60°C for FHIT, 61°C for CDKN2B, DCC, and HPRT1, and 59°C for WNT5B, ADAMTS18, and CMTM5 genes as the annealing step, and 20 s at 72°C. Each PCR reaction was done in triplicates. After completing the polymerase cycle, a melting curve analysis was performed to identify the non-specific PCR products and primer dimer formation. The relative expression data of the genes relative to the internal control gene was obtained using the \(2^{−ΔΔCT)}\) method (Livak method). The fold changes were further converted to the log 10 scale. Then, the mean values of the relative fold changes in the patient group and healthy controls were calculated, analyzed, and compared.

2.4 | Statistical analysis

The value of ΔCts was analyzed for normality of distribution by the Shapiro–Wilk test. For normally distributed data, mean and SD were calculated. Additionally, analysis of variance was used to compare different groups under investigation. Non-normally distributed data were analyzed by non-parametric tests, and medians and ranges were compared using Mann–Whitney test. All data analyses were carried out using the SPSS 18 software and p < 0.05 was considered statistically significant.

3 | RESULTS

This study was conducted on 130 patients with MPNs including 52 patients with positive BCR-ABL1 mutation and 78 with negative BCR-ABL1 mutation (49 with ET, 20 with PV, and 9 with PMF) along with 51 healthy individuals. The mean age of the participants was 53.2 ± 15 years in the patient group and 48.8 ± 16.4 years in the control group (p = 0.054). The mean age of the patients with PV was

| Groups          | CML (n = 52) | PV (n = 20) | ET (n = 49) | PMF (n = 9) | Healthy controls (n = 51) |
|-----------------|-------------|------------|------------|------------|--------------------------|
| Mean age (year ± SD) | 49.7 ± 12.8 | 63.4 ± 13.9 | 52 ± 15     | 56.7 ± 20.2 | 48.8 ± 16.4              |
| Sex, (male/female) | 24/28       | 12/8       | 24/25      | 5/4        | 26/25                    |
| Mean WBC count (x10^3) | 35.6 ± 21.6 | 18.5 ± 8.9 | 14.9 ± 11.3 | 11.5 ± 9.8 | 7.6 ± 2.8                |
| Mean Hb level (gr/dl) | 11.6 ± 3.4  | 17.6 ± 5.2 | 12.3 ± 6.4 | 12.1 ± 4.8 | 12.4 ± 2.1               |
| Mean platelet count (x10^9) | 390.6 ± 112.8 | 488.2 ± 156.4 | 690.7 ± 321.7 | 365.7 ± 196.3 | 187.3 ± 48.9             |
| Splenomegaly    | 21/52       | 7/20       | 11/49      | 4/9        | -                        |

Abbreviations: CML, chronic myeloid leukemia; ET, essential thrombocythemia; MPNs, myeloproliferative neoplasms; PMF, primary myelofibrosis; PV, polycythemia vera.

* p = 0.09 between the patient and control groups (no significant difference). However, a significant difference was found between the patients with PV and other patient groups in terms of mean age (p = 0.004).
63.4 ± 13.9 years, which was significantly higher compared to other MPN categories and the control group (p = 0.021). The male to female ratio was equal to one in the patient group (Table 2).

3.1 | Gene expression

The results showed the normal ΔCts distribution of CMTM5, DCC, and FHIT (Shapiro–Wilk test: 0.290, 0.291, and 0.159, respectively) in contrast to the non-normal ΔCts distribution of ADAMTS18, WNT5B, and CDKN2B (Shapiro–Wilk test: 0.027, 0.001, and 0.005, respectively). In the following sections, the data have been presented as mean ± SD for the normally distributed data and as median (range) or box-plot chart for the non-normally distributed data. p-values have been calculated based on the data type (please refer to the statistics section). Expression fold change (EFC) shows the extent of downregulation (values less than one) and upregulation (values more than one).

Gene expression profiles of ADAMTS18, CMTM5, CDKN2B, DCC, FHIT, and WNT5B mRNAs in all the patients and controls have been presented in Table 3. The results revealed a significant downregulation in CMTM5 (EFC = 0.66) and DCC (EFC = 0.65) genes and a non-significant upregulation of ADAMTS18, CDKN2B, FHIT, and WNT5B genes compared to the control group.

Detailed analysis of the expression changes has been presented in Table 4 (parametric data) and Figure 1 (non-parametric data). The results indicated the significant downregulation of DCC expression in all the subtypes of MPN compared to the control group. Interestingly, CMTM5 expression showed a 1.22-fold upregulation in the patients with PMF compared to other MPN categories, and the difference was statistically significant. However, it presented no significant upregulation compared to the control group. No significant difference was observed among the MPN categories with respect to the expression of other genes including ADAMTS18, CDKN2B, FHIT, and WNT5B (Table 4 and Figure 1).

In the next step, we tried to explore the relationship between gene expression and age and sex. In this study, the participants were divided into lower and higher than 60 years age groups and were compared concerning gene expression. The results revealed no significant difference in this regard in the patient and control groups. However, a statistically significant difference was found in the means of delta CMTM5 and delta DCC between the individuals younger than 60 years in the MPN and control groups (Table 5). In the individuals aged below 60 years, both genes showed
downregulation in the patients with MPNs (EFC = 0.66 and 0.64 for CMTM5 and DCC genes, respectively) (Table 5). Nevertheless, the results demonstrated no significant difference between the two age categories in the patient and control groups regarding ADAMTS18, CDKN2B, FHIT, and WNT5B (Table 5).

A comparison of the sex-related gene expression in the study population has been depicted in Table 6. The male and female participants in the control and MPN groups were separately compared with regard to delta CT. The results only showed a borderline difference between the male participants in the control and MPN groups regarding delta DCC (EFC = 0.69, p = 0.05). On the other hand, the results indicated a significant downregulation in the expressions of both CMTM5 and DCC genes in the female patients compared to the controls (Table 6).

The BCR-ABL1 mutation effect has been shown in Table 7 (normally distributed data) and Figure 2 (non-normal distributed data). The positive BCR-ABL1 mutation led to the overexpression of the WNT5B gene (EFC = 1.6, p = 0.048). However, no significant difference was detected among other genes regarding the BCR-ABL1 mutation status.

| TABLE 4 Gene expression changes in different MPN categories |
|---------------------------------------------------------------|
| **N** | **Mean** | **Std. deviation** | **Expression fold change** | **p-Value** |
| CMTM5 | | | | |
| ET | 49 | -2.761 ± 1.080 | 0.599 | 0.003 |
| PV | 20 | -3.134 ± 1.277 | 0.775 | |
| CML | 52 | -2.823 ± 1.235 | 0.625 | |
| MF | 9 | -3.798 ± 1.088 | 1.229 | |
| Control | 51 | -3.501 ± 1.061 | | |
| Total | 181 | -3.080 ± 1.185 | | |
| DCC | | | | |
| ET | 49 | 4.604 ± 1.163 | 0.678 | 0.024 |
| PV | 20 | 4.619 ± 1.428 | 0.672 | |
| CML | 52 | 4.802 ± 1.178 | 0.591 | |
| MF | 9 | 4.262 ± 1.465 | 0.860 | |
| Control | 51 | 4.045 ± 1.111 | | |
| Total | 181 | 4.488 ± 1.225 | | |
| FHIT | | | | |
| ET | 49 | -1.244 ± 1.500 | 1.019 | 0.835 |
| PV | 20 | -1.431 ± 1.767 | 1.160 | |
| CML | 52 | -1.436 ± 1.437 | 1.164 | |
| MF | 9 | -1.693 ± 1.003 | 1.391 | |
| Control | 51 | -1.216 ± 1.282 | | |
| Total | 181 | -1.334 ± 1.425 | | |

FIGURE 1 Box-plot chart of delta CT in ADAMTS18, WNT5B, and CDKN2B in patient and control groups. The color legend includes a p-value between the categories based on Mann-Whitney U-test. MPNs, myeloproliferative neoplasms (n = 130); CML, chronic myeloid leukemia (n = 52); ET, essential thrombocythemia (n = 49); PMF, primary myelofibrosis (n = 9); PV, polycythemia vera (n = 20)
| Non-parametric factor | N  | Delta CT in the MPN group | p-Value in the MPN group | Delta CT in the control group | EFC | p-Value between the MPN and control groups |
|----------------------|----|---------------------------|--------------------------|-------------------------------|-----|--------------------------------------------|
|                      |    | Median | Min | Max |                      | Median | Min | Max |                      |     |                        |
| ADAMTS18             | 130| 3.37   | 0.68| 6.48| 0.379               | 3.77   | 0.53| 6.70| 1.252               | 0.576| 0.249                    |
| Age <60 years        | 86 | 3.13   | 0.70| 6.48| 0.379               | 3.77   | 0.53| 6.70| 1.252               | 0.576| 0.249                    |
| Age ≥60 years        | 44 | 3.38   | 0.68| 6.29| 0.379               | 4.13   | 1.26| 5.37| 1.176               | 0.576| 0.249                    |
| Total                | 130| 3.37   | 0.68| 6.48| 0.379               | 3.85   | 0.53| 6.70| 1.222               | 0.576| 0.249                    |
| WNT5B                | 130| 2.90   | -1.17| 6.70| 0.414               | 3.37   | -0.68| 5.33| 1.370               | 0.221| 0.221                    |
| Age <60 years        | 86 | 2.76   | -1.17| 6.31| 0.414               | 3.37   | -0.68| 5.33| 1.370               | 0.221| 0.221                    |
| Age ≥60 years        | 44 | 3.25   | -0.67| 6.70| 0.414               | 3.04   | 0.43 | 5.33| 0.948               | 0.903| 0.221                    |
| Total                | 130| 2.90   | -1.17| 6.70| 0.414               | 3.26   | -0.68| 5.53| 1.218               | 0.903| 0.221                    |
| CDKN2B               | 130| 3.34   | 0.92 | 6.75| 0.372               | 3.67   | 1.03| 6.41| 0.919               | 0.652| 0.652                    |
| Age <60 years        | 86 | 3.53   | 1.32 | 6.75| 0.372               | 3.67   | 1.03| 6.41| 0.919               | 0.652| 0.652                    |
| Age ≥60 years        | 44 | 3.18   | 0.92 | 5.95| 0.372               | 4.24   | 1.15 | 6.24| 1.383               | 0.263| 0.263                    |
| Total                | 130| 3.34   | 0.92 | 6.75| 0.372               | 3.87   | 1.03| 6.41| 1.044               | 0.263| 0.263                    |

| Parametric factors   | MPN number | Delta CT in the MPN group (Mean ± SD) | p-Value in the MPN group | Mean of delta CT in the control group | EFC | p-Value between the MPN and control groups |
|----------------------|------------|----------------------------------------|--------------------------|----------------------------------------|-----|--------------------------------------------|
|                      |            | Median | Min | Max |                      | Median | Min | Max |                      |     |                        |
| CMTM5                |            | -2.940| 1.095|    | 0.740                   | -3.543 |     |     | 0.659               | 0.007| 0.007                    |
| Age <60 years        | 86         | -2.940| 1.095|    | 0.740                   | -3.543 |     |     | 0.659               | 0.007| 0.007                    |
| Age ≥60 years        | 44         | -2.866| 1.380|    | 0.740                   | -3.401 |     |     | 0.690               | 0.165| 0.165                    |
| Total                | 130        | -2.915| 1.194|    | 0.740                   | -3.501 |     |     | 0.666               | 0.165| 0.165                    |
| DCC                  |            | 4.676 | 1.191|    | 0.855                   | 4.033  |     |     | 0.640               | 0.007| 0.007                    |
| Age <60 years        | 86         | 4.676 | 1.191|    | 0.855                   | 4.033  |     |     | 0.640               | 0.007| 0.007                    |
| Age ≥60 years        | 44         | 4.634 | 1.310|    | 0.855                   | 4.073  |     |     | 0.678               | 0.143| 0.143                    |
| Total                | 130        | 4.662 | 1.227|    | 0.855                   | 4.045  |     |     | 0.652               | 0.143| 0.143                    |
| FHIT                 |            | -1.358| 1.447|    | 0.807                   | -1.408 |     |     | 0.965               | 0.856| 0.856                    |
| Age <60 years        | 86         | -1.358| 1.447|    | 0.807                   | -1.408 |     |     | 0.965               | 0.856| 0.856                    |
| Age ≥60 years        | 44         | -1.425| 1.559|    | 0.807                   | -0.760 |     |     | 1.591               | 0.135| 0.135                    |
| Total                | 130        | -1.381| 1.480|    | 0.807                   | -1.216 |     |     | 1.120               | 0.135| 0.135                    |

Abbreviation: EFC, expression fold change.
Bold indicates significant p value (<0.05).
DISCUSSION

According to the 2016 classification system of hematologic malignancies, based on the BCR-ABL1 mutation, MPNs can be divided into two groups; that is, BCR-ABL1+ (CML) and BCR-ABL1- (PV, ET, and PMF). DNA methylation, the most common epigenetic change, plays a significant role in the pathogenesis of different hematologic malignancies including MPNs. Totally, two abnormal methylation patterns have been found in some cancers, resulting in a decrease or an increase in transcription. Although the exact mechanism leading to abnormal DNA methylation is unknown, chronic inflammation, oxidative stress, alcohol, and aging have been claimed to contribute to the process. Evidence has indicated that heterogeneous genes are affected in this process. After deeply investigating the previous studies and the GEO databases, the six genes presented in the current study (five tumor suppressors and one oncogene) are linked to hematologic disorders. The current study has evaluated the association between DNA methylation and gender difference with the following results:

**Table 6: Comparison of males and females regarding gene expression**

| Gene | Male | Delta CT (SD) | p-Value in males | Female | Delta CT (SD) | p-Value in females |
|------|------|---------------|------------------|--------|---------------|--------------------|
| ADAMTS18 | Patient | 3.32 ± 0.78 | 0.072 | 1.32 | 3.39 ± 0.87 | 0.753 |
| | Control | 4.04 ± 0.53 | 0.67 | 0.53 | 4.13 ± 0.87 | 0.753 |
| | Total | 3.66 ± 0.53 | 0.67 | 0.53 | 4.13 ± 0.87 | 0.753 |
| WNT5B | Patient | 2.83 ± 1.06 | 0.457 | 1.28 | 3.13 ± 1.17 | 0.933 |
| | Control | 3.26 ± 0.43 | 0.87 | 6.53 | 3.10 ± 0.68 | 0.503 |
| | Total | 3.01 ± 0.67 | 0.87 | 6.53 | 3.12 ± 1.17 | 0.622 |
| CDKN2B | Patient | 3.62 ± 0.92 | 0.763 | 1.04 | 3.21 ± 1.50 | 0.834 |
| | Control | 3.77 ± 1.03 | 0.87 | 6.41 | 3.87 ± 1.15 | 0.525 |
| | Total | 3.66 ± 0.92 | 0.87 | 6.41 | 3.87 ± 1.15 | 0.525 |

**Table 7: Comparison of the mean gene expression in the patients with and without the BCR-ABL1 mutation**

| Gene | BCR-ABL1 chromosome | N | Mean delta CT (SD) | p-Value | Expression fold |
|------|---------------------|---|-------------------|---------|----------------|
| CMTM5 | BCR-ABL1− | 78 | −2.976 ± 1.169 | 0.475 | 0.695 |
| | BCR-ABL1+ | 52 | −2.823 ± 1.235 | 0.625 | 0.666 |
| | Total | 130 | −2.915 ± 1.194 | 0.666 | 0.666 |
| DCC | BCR-ABL1− | 78 | 4.568 ± 2.58 | 0.289 | 0.696 |
| | BCR-ABL1+ | 52 | 4.802 ± 1.78 | 0.591 | 0.591 |
| | Total | 130 | 4.662 ± 1.227 | 0.652 | 0.652 |
| FHIT | BCR-ABL1− | 78 | −1.344 ± 1.517 | 0.728 | 1.092 |
| | BCR-ABL1+ | 52 | −1.436 ± 1.437 | 1.164 | 1.164 |
| | Total | 130 | −1.381 ± 1.480 | 1.120 | 1.120 |

**Abbreviation:** EFC, expression fold change.

Bold indicates significant p value (<0.05).
one oncogene) were found to be more likely to be affected by the methylation process. In the present study, therefore, the ECF of the selected genes was explored as the first step to explore the possible association between these genes and MPN pathogenesis and clinical outcome.\textsuperscript{7,16,17,21,22} To the best of our knowledge, the present study is one of the few studies exploring the expression levels of some tumor suppressors and oncogenes in a population of patients with MPNs.

Among the five genes mainly known as tumor suppressor genes, two (CMTM5 and DCC) showed a significant downregulation. These genes are involved in many biological processes in the human body. For instance, CMTM5 has a tumor inhibitory function, an immune system modulation capability, and an active role in the male reproductive system.\textsuperscript{16,22} Many cancers such as myeloid leukemia, prostate cancer, and cervical cancer are associated with the downregulation of this gene.\textsuperscript{16,22,23} Another tumor suppressor gene, which is primarily known as "deleted in colorectal carcinoma" or DCC, encodes the netrin receptor protein and acts as both a conditional oncogene and a conditional tumor suppressor.\textsuperscript{24,25} After the initial detection of this gene in colorectal cancer, its role has been rapidly highlighted in other malignancies. To date, the effect of this gene has been recognized in many malignancies including hematologic malignancies. The absence of DCC is a prognostic factor in AML and MDS pathogenesis. Additionally, tumor suppression and metastasis suppression have been reported with the restitution of normal DCC function. Inokuchi et al. disclosed that the absence of the DCC gene contributed to the pathogenesis of MDS and AML and might worsen the AML prognosis.\textsuperscript{25,26} In another study on a rare form of MPN; that is, chronic neutrophilic leukemia, the role of DCC was investigated in the MPN subcategories. The results demonstrated that DCC heterozygote patients had a shorter latency period compared to those with the myeloproliferative disease.\textsuperscript{27} Furthermore, less than 30% DCC gene absence or downregulation was reported in CML cases.\textsuperscript{28,29} In colorectal cancer, the absence or downregulation of this gene was associated with poor prognosis and an increased rate of metastasis and progression from the benign to a malignant form of colorectal tumors. It also played an important role in the loss of homogeneity, which contributed to poor prognosis in these tumors.\textsuperscript{30–33} Up to now, little attention has been paid to the role of this gene and its expression. Considering the 0.652 DCC downregulation among the cases with MPNs (Table 3) that was significant compared to the control group ($p = 0.002$), the influence of this gene
and its related functions is suggested to be taken into account in some pathogeneses and phenotypic presentations of MPNs.

The role of CMT5M in hematological and non-hematological malignancies has been investigated in different studies, indicating this gene as a potent tumor suppressor gene.\textsuperscript{16,22,23,34–37} This gene encodes a member of the chemokine-like factor superfamily and exhibits tumor suppressor features. Niu Jihong et al. reported the downregulation of CMT5 M in the AML cases and its return to the normal level with successful treatment.\textsuperscript{25} The main role of CMT5 M in malignancies is related to the induction of apoptosis and cell-cycle arrest. Therefore, its down-regulation leads to the survival of malignant cells.\textsuperscript{35,37,38} The present study findings showed the significant downregulation (near 40%) of this gene in MPNs, which is probably a contributing factor to the pathogenesis of this proliferative neoplasm. Yet, more precise investigations are required due to the multifactorial nature of this category of hematological malignancies.

The current study results revealed the upregulation of four genes including ADAMTS18, CDKN2B, and FHIT as tumor suppressors and WNT5B as an oncogene in the patients. However, no significant difference was found in the control group in this regard. Although these genes have multiple functions, they all have tumor suppressor activities with different mechanisms.\textsuperscript{39–42} Their normal functions decrease their role in the pathogenesis of MPNs.

In the current research, the subcategories of MPNs were reanalyzed to obtain more details about the downregulation of these genes. Although the PMF cases had a lower DCC EFC, all the subcategories of MPNs showed downregulations with EFCs ranging from 0.6 to 0.8 (Table 4). The CMT5 gene had a different pattern of EFC among the MPN subcategories. This gene was downregulated in the patients with ET, PV, and CML, but upregulated in those with PMF. No similar studies were found to compare the results obtained in the patients with PMF. Hence, explaining this finding needs further investigations, and the results should be interpreted in the context of PMF pathophysiology and the wide range of CMT5M functions such as the immune system. Age is one of the possible factors in changes in gene expression. Thus, the EFC was analyzed in two age categories. Interestingly, the results indicated that both DCC and CMT5M genes were significantly downregulated in the patients aged below 60 years compared to the age-matched controls. This implied that the downregulation of DCC and CMT5M played a more important role in the pathogenesis of MPNs at ages below 60 years. However, the difference between the two age groups was on the borderline and, consequently, should be reinvestigated in future studies with larger sample sizes (Table 5). Some other studies in different contexts and malignancies did not report any correlation between age and the gene expression of CMT5M.\textsuperscript{43} As expected, the present study findings also showed the ineffectiveness of age in the gene expressions of ADAMTS18, CDKN2B, FHIT, and WNT5B.

Furthermore, the role of sex in the expression of these genes was evaluated in this study. Based on the results, females showed a more prominent downregulation of DCC and CMT5M in the setting of MPNs (Table 6). However, none of these genes had a significantly different expression between the males and females in the control group. Thus, the sex difference in the expression rate was ruled out. The role of the female sex was more prominent in the CMT5M gene expression. In other words, in the female patients with MPNs, the maximum downregulation among all the genes was observed for CMT5M with EFC = 0.436. Nonetheless, the male patients showed no significant difference in the expression of this gene (male EFC = 0.858; p-value with the control group =0.36). This significant female predominance in CMT5M downregulation was not suggested by other studies on CMT5M expression in hematological or non-hematological malignancies.\textsuperscript{22,23,43}

Considering the DCC gene, the expression change was significant in the female patients. Male patients also showed a borderline significant downregulation compared to the male controls (male EFC = 0.698, p = 0.05; female EFC = 0.570, p = 0.015). This highlighted the role of CMT5M in the pathogenesis of MPN, particularly in female patients.

In the current investigation, the CML patients presented the overexpression of the WNT5B gene (p = 0.048). Considering the non-normal distribution of the data represented in Figure 2, Mann–Whitney test was employed. The results revealed that EFC was close to one among the patients with negative BCR-ABL1 mutation, which implied no change in gene expression. Nevertheless, the positive cases had the highest value of overexpression (EFC = 1.6). It seemed that the BCR-ABL1 mutation had a synergistic effect on the expression of the WNT5B gene. The overexpression of this gene, as an oncogene, has been reported in many studies, some of which indicated BCR-ABL1 as an aggravating factor for its overexpression.\textsuperscript{44,45} This gene could promote cell migration and invasion, eventually leading to poor outcomes\textsuperscript{17,46} thus, it may be reasonable to investigate the role of WNT5B overexpression in the prognosis of BCR-ABL1+ cases.

5 | CONCLUSION

The present study was one of the few studies investigating the role of tumor suppressors and oncogenes in MPNs. MPNs and their subcategories exhibited significant CMT5M and DCC downregulations, which were pronounced in females as well as in individuals aged below 60 years. Additionally, the WNT5B oncogene was overexpressed in the CML cases (BCR-ABL1+ cases). Yet, further clinical studies are required to be conducted on EFC in tumor suppressors and oncogenes so as to provide new insights into the pathogenesis of patients with MPNs.

ACKNOWLEDGEMENTS

The authors would like to thank Ms. A. Keivanshekouh at the Research Consultation Center (RCC) of Shiraz University of Medical Sciences for improving the use of English in the manuscript.

CONFLICT OF INTEREST

None declared.
INFORMED CONSENT
The study was explained to the patients and their informed consent forms were obtained.

DECLARATION
To the best of our knowledge, the present study is one of the few studies exploring tumor suppressor genes or oncogenes in a population of MPN patients (Philadelphia positive & Philadelphia negative). This study aimed to evaluate the expression of multiple genes (five tumor suppressors and one oncogene) and to highlight the relationship between these genes and some patients’ features. Two tumor suppressor genes, CMTM5 and DCC, were downregulated in patients with MPN, especially in females and younger cases. Known oncogenesis, WNT5B, is overexpressed by up to 1.6 folds in Philadelphia positive MPNs. The findings pertaining to oncogenes and tumor suppressors may illuminate a new pathway in the MPN pathogenesis or a new index for MPN treatment and outcome.

DATA AVAILABILITY STATEMENT
The author elects not to share data.

ORCID
Elham Abedi  https://orcid.org/0000-0002-0304-1452
Mehran Karimi  https://orcid.org/0000-0001-8555-1001
Ramin Yaghobi  https://orcid.org/0000-0002-9812-8621
Hamid Mohammadi  https://orcid.org/0000-0002-6606-2795
Sezaneh Haghpahan  https://orcid.org/0000-0002-8666-2106
Mohamad Moghadam  https://orcid.org/0000-0001-8290-7332
Mani Ramzi  https://orcid.org/0000-0003-2283-5036

REFERENCES
1. Langabeer SE, Andrikovics H, Asp J, et al. Molecular diagnostics of myeloproliferative neoplasms. *Eur J Haematol*. 2015;95(4):270-279.
2. Nguyen-Khac F, Couronne L, Eclache V. Chromosomal abnormalities in transformed ph-negative myeloproliferative neoplasm are independent of the JAK2 and the TET2 statuses. *Blood*. 2009;114(22):2900. doi:10.1182/blood.v114.22.2900.2900
3. Zhang S-J, Rampal R, Manshouri T, et al. Genetic analysis of patients with leukemic transformation of myeloproliferative neoplasms shows recurrent SRSF2 mutations that are associated with adverse outcome. *Blood*. 2012;119(19):4480-4485.
4. Lin CY, Ho CM, Tamamyan G, Yang SF, Peng CT, Chang JG. Validating the sensitivity of high-resolution melting analysis for JAK2 V617F mutation in the clinical setting. *J Clin Lab Anal*. 2016;30(6):838-844.
5. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood*. 2016;127(20):2391-2405. doi:10.1182/blood-2016-03-643544
6. Barbui T, Thiele J, Gisslinger H, et al. The 2016 WHO classification and diagnostic criteria for myeloproliferative neoplasms: document summary and in-depth discussion. *Blood Cancer J*. 2018;8(2):1-11.
7. Găman M-A, Cosma M-A, Dobrăcă E-C, Crețoiu SM, Găman AM, Diaconu CC. Liquid biopsy and potential liquid biopsy-based biomarkers in Philadelphia-negative classical myeloproliferative neoplasms: a systematic review. *Life*. 2021;11(7):677.
8. Gaman AM, Moisa C, Diaconu CC, Gaman AM. Crosstalk between oxidative stress, chronic inflammation and disease progression in essential thrombocytopenia. *Rev Chim*. 2019;70(10):3486-3489.
9. Holyoake TL, Vetrie D. The Chronic Myeloid Leukemia Stem Cell: Stemming the Tide of Persistence. 2017;129(12):1595-1606.
10. Radič J, Yeung C, Wu D. New approaches to molecular monitoring in CML (and other diseases). *Blood*. 2019;134(19):1578-1584.
11. Hehlmann R. Chronic Myeloid Leukemia in 2020. *HemaSphere*. 2020;4(5):e468.
12. Lin J, Yao DM, Qian J, et al. Methylation status of Fragile Histidine Triad (FHIT) gene and its clinical impact on prognosis of patients with myelodysplastic syndrome. *Leuk Res*. 2008;32(10):1541-1545.
13. Pérez C, Pascual M, Martín-Subero JI, et al. aberrant DNA methylation profile of chronic and transformed classic Philadelphia-negative myeloproliferative neoplasms. *Haematologica*. 2013;98(9):1414-1420.
14. Jelinek J, Mannari R, Issa J-P. Identification of 41 novel promotor-associated CpG Islands methylated in leukemias. *Blood*. 2004;104(11):1126. doi:10.1182/blood.v104.11.1126.1126
15. Lu D, Zhao Y, Tatwara R, et al. Activation of the Wnt signaling pathway in chronic lymphocytic leukemia. *Proc Natl Acad Sci USA*. 2004;101(9):3118-3123.
16. Xiao Y, Yuan Y, Zhang Y, et al. CMTM5 is reduced in prostate cancer and inhibits cancer cell growth in vitro and in vivo. *Clin Transl Oncol*. 2015;17(6):431-437.
17. Wang SH, Chang JS, Hsiao JR, et al. Tumour cell-derived WNT5B modulates in vitro lymphangiogenesis via induction of partial endothelial-mesenchymal transition of lymphatic endothelial cells. *Oncogene*. 2017;36(11):1503-1515.
18. Rampal R, Al-Shahrour F, Abdel-Wahab O, et al. Integrated genomic analysis illustrates the central role of JAK-STAT pathway activation in myeloproliferative neoplasm pathogenesis. *Blood*. 2014;123(22):e123-e133.
19. Wong WJ, Hasserjian RP, Pinkus GS, Breyfogle LJ, Mullally A, Pozdnjakova O. JAK2, CALR, MPL and ASXL1 mutational status correlates with distinct histological features in Philadelphia chromosome-negative myeloproliferative neoplasms. *Haematologica*. 2018;103(2):e63-e68.
20. Moisa C, Gaman MA, Diaconu CC, Gaman AM. Oxidative stress levels, JAK2V617F mutational status and thrombotic complications in patients with essential thrombocytopenia. *Rev Chim*. 2019;70(8):2822-2825.
21. Takada S, Morita K, Hayashi K, et al. Methylation status of Fragile Histidine Triad (FHIT) gene and its clinical impact on prognosis of patients with multiple myeloma. *Eur J Haematol*. 2005;75(6):S50-S51.
22. Niu J, Li H, Zhang Y, et al. aberrant expression of CKLF-like MARVEL Transmembrane Member 5 (CMTM5) by promoter methylation in myeloid leukemia. *Leuk Res*. 2011;35(6):771-776.
23. Ma Y, Shi JF, Qiu HY, et al. [Pathophysiologic mechanism of CMTM5 low expression in multiple myeloma progression]. *Zhonghua Xue Ye Xue Za Zhi*. 2019;40(1):58-62.
24. Arakawa H. Nethrin-1 and its receptors in tumorogenesis. *Nat Rev Cancer*. 2004;4(12):978-987.
25. Inokuchi K, Miyake K, Takahashi H, Dan K, Nomura T. DCC protein expression in hematopoietic cell populations and its relation to leukogenesis. *J Clin Invest*. 1996;97(3):852-857.
26. Inokuchi K, Yamaguchi H, Hanawa H, et al. Loss of DCC gene expression is of prognostic importance in acute myelogenous leukaemia. *Clin Cancer Res*. 2002;8(6):1882-1888.
27. Inokuchi K, Inami M, Wang Y, et al. The DCC heterozygote reduces the latency period and changes the disease phenotype of myeloproliferative disease in p230BCR/ABL-expressing mice. *Blood*. 2004;104(11):2968. doi:10.1182/blood.v104.11.2968.2968
28. Porfiri E. DCC (deleted in colorectal cancer) inactivation in hematological malignancies. *Leukemia & Lymphoma*. 1995;18(1-2):69-72.
29. Miyake K, Inokuchi K, Nomura T. Expression of the DCC gene in human hematological malignancies. *Leuk Lymphoma*. 1994;16(1-2):13-18.
30. Duman-Scheel M. Deleted in Colorectal Cancer (DCC) pathfind-
ing: axon guidance gene finally turned tumor suppressor. Curr Drug
Targets. 2012;13(11):1445-1453.
31. Sefrioui D, Vermeulin T, Blanchard F, et al. Copy number vari-
tions in DCC/18q and ERBB2/17q are associated with disease-
free survival in microsatellite stable colon cancer. Int J Cancer.
2017;140(7):1653-1661.
32. Chen HJ, Wei Z, Sun J, et al. A recellularized human colon model
identifies cancer driver genes. Nat Biotechnol. 2016;34(8):845-851.
33. Jiang HW, Wang J, Li HJ, Peng JK, Gao XP, Chen F. Influence of the
DCC gene on proliferation and carcioembryonic antigen expres-
sion in the human colorectal cancer cell line SW1116. Genet Mol
Res. 2015;14(3):10273-10280.
34. Xu T, Li J, Xiao YB, Liu ZH, Li Q, Wang XF. [CMTM5 inhibits the
tumor cell behavior of prostate cancer by downregulation of HER2].
Beijing da xue xue bao Yi xue ban = Journal of Peking University
Health Sciences. 2010;42(4):386-390.
35. Shao L, Guo X, Plate M, et al. CMTM5-v1 induces apoptosis in cervical
carcinoma cells. Biochem Biophys Res Comm. 2009;379(4):866-871.
36. Guo X, Li T, Wang Y, et al. CMTM5 induces apoptosis of pancreatic
cancer cells and has synergistic effects with TNF-alpha. Biochem
Biophys Res Comm. 2009;387(1):139-142.
37. Cai B, Xiao Y, Li Y, Zheng S. CMTM5 inhibits renal cancer cell growth
through inducing cell-cycle arrest and apoptosis. Oncology Letters.
2017;14(2):1536-1542.
38. Zhang H, Nan X, Li X, et al. CMTM5 exhibits tumor suppressor activ-
ity through promoter methylation in oral squamous cell carcinoma.
Biochem Biophys Res Comm. 2014;447(2):304-310.
39. Malak CA, Elghanam DM, Elbossaty WF. FHIT gene expression in
acute lymphoblastic leukemia and its clinical significance. Asian Pac
J Cancer Prev. 2015;16(18):8197-8201.
40. Kim HS, Kitano Y, Mori M, et al. The novel secreted factor MIG-
18 acts with MIG-17/ADAMTS to control cell migration in
Caenorhabditis elegans. Genetics. 2014;196(2):471-479.
41. Kiss DL, Baez W, Huebner K, Bundschuh R, Schoenberger DR. Impact
of FHIT loss on the translation of cancer-associated mRNAs. Mol
Cancer. 2017;16(1):179.
42. Sun Y. [The relationship between FHIT gene promoter methyla-
tion and lung cancer risk: a meta-analysis]. Zhongguo Fei Ai Za Zhi.
2014;17(3):233-237.
43. Xu G, Dang C. CMTM5 is downregulated and suppresses tumour
growth in hepatocellular carcinoma through regulating PI3K-AKT
signalling. Cancer Cell Int. 2017;17(1):113.
44. Saitoh T, Kato M. Expression and regulation of WNT5A and
WNT5B in human cancer: up-regulation of WNT5A by TNFα in
MKN45 cells and up-regulation of WNT5B by β-estradiol in MCF-7
cells. Int J Mol Med. 2002;10(3):345-349.
45. Sercan H, Demirtaş Pehlivan M, Papur O, Ates H, Sercan Z.
Induction of apoptosis increases expression of non-canonical WNT
genes in myeloid leukemia cell lines. Oncol Rep. 2008;18:1563-1569.
46. Harada T, Yamamoto H, Kishida S, et al. Wnt5b-associated exo-
somes promote cancer cell migration and proliferation. Cancer Sci.
2017;108(1):42-52.

How to cite this article: Abedi E, Karimi M, Yaghobi R, et al.
Oncogenic and tumor suppressor genes expression in
myeloproliferative neoplasms: The hidden side of a complex
pathology. J Clin Lab Anal. 2022;36:e24289. doi:10.1002/
jcla.24289