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**ORIGINAL ARTICLE**

# Expression and methylation status of *BTG2*, *PPP1CA*, and *PEG3* genes in colon adenocarcinoma cell lines: promising treatment targets

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## ABSTRACT

**Aim**: This study investigated the association between methylation status and expression levels of *BTG2*, *PPP1CA*, and *PEG3* genes in colon cancer.

**Background**: Aberrant DNA methylation is one of the most important epigenetic modifications in the development of cancer. Evidence indicates that hypermethylation of various tumor suppressor genes could be a potential mechanism of colon tumorigenesis.

**Methods**: The expression levels of *BTG2*, *PPP1CA*, and *PEG3* genes were evaluated in HT-29/219, HCT116, SW48, SW742, SW480, and LS180 cell lines using quantitative Real-Time PCR. The methylation status of *BTG2* and *PPP1CA* was determined by methylation-specific PCR (MSP) method, and the methylation pattern of PEG3 was evaluated by bisulfite sequencing PCR (BSP). To investigate the effect of methylation on the expression of these genes, all colon cancer cell lines were treated by 5-Azacitidine (5-Aza) and/or Trichostatin A (TSA).

**Results:** The expression levels of *BTG2*, *PPP1CA*, and *PEG3* were highly heterogeneous and quantitatively correlated to their promoter methylation status in the studied colon cancer cell lines. Treatment by 5-Aza and/or TSA increased the expression of the above-named genes in colon cancer cell lines.

**Conclusion**: Overall, it seems that *BTG2*, *PPP1CA*, and *PEG3* act as tumor suppressor genes in colon cancer, and methylation is a potential mechanism for their loss of expression. Therefore, these genes may be considered as suitable targets for demethylation approaches and, eventually, colon cancer treatment. Combined treatment by 5-Aza and TSA may be a promising therapeutic strategy for colon cancer treatment. Further studies may contribute to confirm these results.

**Keywords**: Colon cancer, *BTG2*, *PPP1CA*, *PEG3*, DNA.

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## Introduction

Colorectal cancer (CRC) is the third most prevalent cancer and has become a big concern around the world (1). Lifestyle, micro-environmental factors, genetic and epigenetic modifications are considered in the etiology of this type of cancer (2, 3). DNA, histone proteins, and non-coding RNAs are affected by epigenetic modifications (4, 5). DNA methylation is one such change that plays an important role in growth and evolution processes in the physiological condition. DNA methyltransferases constitute a family of enzymes which catalyze methyl transfer from S-adenosyl methionine (SAM) to cytosine residues of DNA (6). Aberrant methylation leads to gene expression modification and ultimately results in different diseases, like cancer (4, 7), autoimmune diseases (8), high blood pressure (9), atherosclerosis

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(10), cardiovascular diseases (11), mental disorders (12), diabetes, and obesity (13). Hypermethylation of DNA repair and tumor suppressor genes as well as hypomethylation of oncogenes are considered as epigenetic biomarkers for CRC (4). Continuing efforts to discover more about the epigenetic changes in various genes related to CRC may lead to identifying new epigenetic biomarkers for the diagnosis, prognosis, and prediction of this disease.

B-cell translocation gene 2 (*BTG2*) is the first identified member of the BTG/TOB family; it is located on chromosome 1q32.1 and encodes a 158 amino acid protein (14). As a member of the anti-proliferative (APRO) gene family, *BTG2* plays a critical role in different processes, like cell differentiation, DNA repair promotion, cell proliferation inhibition, and apoptosis induction (15). According to the available reports, *BTG2* is downregulated and acts as a tumor suppressor gene in various types of cancers, such as lung (14), breast (16), prostate (17), liver (18), kidney (19), melanoma (20), larynx (21), and gastric cancers (22). Moreover, it is a target of miR-934. *BTG2* knockdown by miR-934 overexpression in colorectal cancer leads to cell proliferation, migration, invasion, and angiogenesis (23).

Paternally expressed gene 3 (*PEG3*) is a Kruppel-type (C2H2) zinc-finger protein with molecular weight of 140 kDa. This protein is encoded by an imprinted gene located on 19q13.4 and expressed in tissue of the brain, testis, ovary, and placenta. PEG3 plays a key role in the control of fetal growth, nutritional behavior, and mammalian reproduction (24, 25). Evidence has confirmed the tumor suppressor activity of *PEG3* and its methylation-induced downregulation in different types of cancer, like ovarian, cervical, breast, and glioma cancers (24, 26-28). The low expression of *PEG3* mRNA and its role in the occurrence and prognosis of colon cancer has also been reported (29).

Serine/threonine specific protein phosphatase 1 (PP1) is involved in the regulation of different processes like cell division, protein synthesis, neurotransmission, muscle contraction, and glycogen metabolism (30). One of the three subunits of PP1 is Protein Phosphatase 1 Catalytic Subunit Alpha (*PPP1CA*). Reduced *PPP1CA* expression is reported in various cancers, including small intestine, prostate, vulva, colorectal, and kidney tumors. The significant allelic loss of *PPP1CA* has also been observed in colorectal and kidney cancers (31). Growth reduction due to *PPP1CA* upregulation and malignancy enhancement due to *PPP1CA* downregulation led to the determination that *PPP1CA* can act as a tumor suppressor (31).

Previous studies have identified a significant association between methylation and reduced expression of the above-named tumor suppressor genes (*BTG2*, *PEG3*, and *PPP1CA*) in various cancers such as breast, ovary, cervix, glioma (24, 26-28), B-cell acute lymphoblastic leukemia (B-ALL) (32), and non-small cell lung cancers (NSCLC) (14). Therefore, it seems that epigenetic modification regulates the expression of these tumor suppressor genes, and their aberrant methylation may lead to tumorigenesis.

To the best of our understanding of the literature, there is yet no study on the association between methylation and expression status of these genes in colorectal cancer. Given the importance of this issue, the present study was designed to evaluate the expression status and methylation pattern of the *BTG2*, *PEG3*, and *PPP1CA* genes in different colon adenocarcinoma cell lines. For this purpose, we used 5-Aza, which is FDA-approved for routine clinical treatment, as a DNA methyltransferase inhibitor (demethylating agent). However, varying results have been obtained for 5-Aza application in several solid tumors. The available evidence indicates there is a synergy between DNA methylation and histone modifications in the early stages of carcinogenesis (33, 34). Therefore, combination therapy with inhibitors of these processes may have better therapeutic effects compared to each drug alone (33). In this regard, we applied TSA as a histone deacetylase inhibitor along with 5-Aza.

## Materials and methods

Six human colon adenocarcinoma cell lines (HT-29/219, HCT116, SW48, SW742, SW480, and LS180) were purchased from the National Cell Bank of Iran (NCBI, Pasteur Institute, Tehran). HCT116, SW48, SW742, HT29/219, and SW480 cells were cultured in RPMI 1640, and LS180 was cultivated in DMEM, containing 10% fetal bovine serum, 100 UmL-1

penicillin, 100 µgmL-1 streptomycin, and 2 mM glutamine in a 5% $CO_2$ humidified incubator at 37 °C.

## 5-Azacitidine and Trichostatin A treatment

Each colon adenocarcinoma cell line was cultured in 6-well microplates. Then, after 24 hours (h), cells were treated with 2.5 µM 5-Aza (dissolved in DMSO) for 72 h. To inhibit histone deacetylation, cell lines were incubated for 24 h in media containing 300 nM of TSA (dissolved in DMSO). For combined inhibition, cells were treated with 5-aza for 3 days, and then TSA was added in the last 24 h of treatment, as previously described (32, 35).

## RNA extraction and real-time quantitative PCR assay

RNA was extracted from cultured cells applying an RNA isolation kit (Roche Applied Science, Germany) according to the manufacturer's guidelines. cDNA was synthesized using a RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Germany). Then, expression levels of BTG2, PPP1CA, and PEG3 genes as well as the internal reference gene (GAPDH) were determined by real-time quantitative PCR (qPCR) assay using ABI 7500 Sequence Detection System (Applied Biosystems, USA) and the specific primers depicted in Table 1. The qPCR reactions were performed in triplicate with a 60-°C annealing temperature and a total cycle number of 40. The qPCR always included a no-template sample as a negative control. The relative expression levels of *BTG2*, *PPP1CA*, and *PEG3* mRNA were normalized to GAPDH mRNA levels and determined using formula $2^{-\Delta\Delta CT}$.

## Methylation analysis

Genomic DNAs of the cultured cells were extracted using the phenol-chloroform extraction method as previously described (36). Then, EpiTect Bisulfite Kit (QIAGEN) was applied to convert unmethylated cytosines to uracils. The methylation status of the *BTG2* and *PPP1CA* genes was determined by methylation specific PCR (MSP) using the bisulfite-converted DNA as the template. The sequences of methylated and unmethylated specific primers for each gene are listed in Table 2, based on previous study (32).

The methylation status of the *PEG3* gene promoter was evaluated in six cell lines using bisulfite sequencing PCR (BSP). The target region of the promoter of the *PEG3* gene, containing 23 CpG sites, was amplified by PCR using Taq DNA Polymerase Master Mix RED (Cat No. A180301) and specific primers (Forward, 5'-TTGTTGTTGTGGTAATCGTAGTTTGATTG-3' and Reverse, 5'-GTATTGAGGTGAGGTGAGTGCGTT-3'). Then, PCR products were sent for sequencing by Bioneer Company.

**Table 1.** Primer sequences applied for quantitative Real-Time PCR assay.

| Gene | Primer sequence | Product size (bp) |
|---|---|---|
| *PPP1CA* | Forward 5′-GCCAGCATCAACCGCATC-3′ | 236 |
| | Reverse 5′-CACAGCAGGTCACACAGC -3′ | |
| *BTG2* | Forward 5′-GAGCCACGGGAAGGGAAC-3′ | 217 |
| | Reverse 5′-CTTGTGGTTGATGCGAATGC-3′ | |
| *PEG3* | Forward 5′- CCTACCCAAGCACCAGTC -3′ | 137 |
| | Reverse 5′- GGAACTGCGTGACACATC -3′ | |
| *GAPDH* | Forward 5´- CGACCACTTTGTCAAGCTCA -3´ | 258 |
| | Reverse 5´- AGGGGTCTACATGGCAACTG -3´ | |

**Table 2.** Specific primers used for methylation-specific PCR, annealing temperatures, and PCR product sizes.

| Gene | Primer sequences | Annealing Temperature | Product Size |
|---|---|---|---|
| *PPP1CA* | MF: 5'-TAGCGAGGTTTCGTGGTC-3' | 50 °C | 156 bp |
| | MR: 5'-ACCGAACTTCCCGAATTTCT-3' | | |
| | UF: 5'-GGGTAGTGAGGTTTTGTGGTT-3' | 55 °C | 156 bp |
| | UR: 5'-ACCAAACTTCCCAAATTTCTCC-3' | | |
| *BTG2* | MF: 5'-TTCGAGTTTTAAAAATGGGC-3' | 46 °C | 175 bp |
| | MR: 5'- CGCTCGCTATCGTCAATA-3' | | |
| | UF: 5'- TAATTTGAGTTTTAAAAATGGGT-3' | 53 °C | 175 bp |
| | UR: 5'- TCACTCACTATCATCAATACT-3' | | |

MF: Methylated Forward, MR: Methylated Reverse, UF: Unmethylated Forward, UR: Unmethylated Reverse.

## Statistical analysis

Data analysis was conducted applying SPSS software version 20 (Chicago, IL). Experiments were analyzed using one-way ANOVA accompanied by Tukey's multiple comparison tests. Data is presented as mean ± SD. Differences with a p-value ≤ 0.05 were considered as statistically significant.

## Results

### *BTG2*, *PPP1CA*, and *PEG3* genes expression is heterogeneous in CRC cell lines

Expression levels of *BTG2*, *PPP1CA*, and *PEG3* genes were evaluated by real-time quantitative PCR assay in six human colon adenocarcinoma cell lines, namely HT-29/219, HCT116, SW48, SW742, SW480, and LS180 (Figure 1). No expression of the *BTG2* and *PPP1CA* genes was observed in the SW48 cell line; therefore, it was selected as the baseline/reference for *BTG2* and *PPP1CA* expression levels (set at 1.0). In comparison with SW48 as a reference, the highest *BTG2* levels were observed in HCT116, LS180, HT29/219 ($p<0.01$), and SW742 ($p<0.05$), respectively. *BTG2* expression was low in SW480 cells, and no significant difference with SW48 as the reference ($p>0.05$) was observed. Similarly, the highest expression level of *PPP1CA* was observed in HCT116 ($p<0.001$). The other four cell lines also had significantly higher levels of *PPP1CA* compared with SW48 ($p<0.01$). *PEG3* gene expression was not observed in the SW480 cell line; accordingly, it was

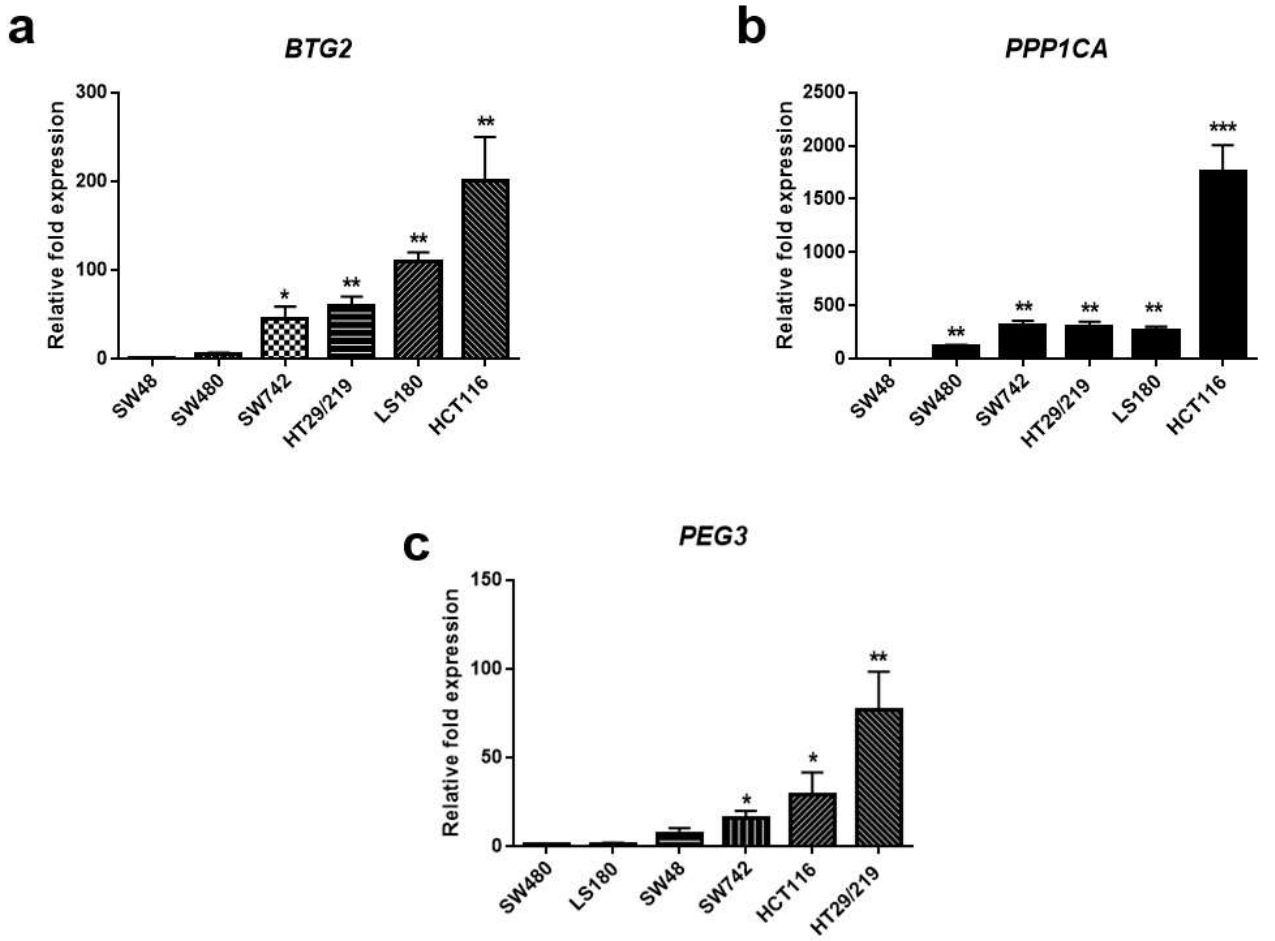


**Figure 1.** The relative expression of *BTG2*, *PPP1CA*, and *PEG3* in colon adenocarcinoma cell lines was determined by quantitative RT-PCR. Expression was normalized to the GAPDH mRNA control. The SW48 cell line was considered as a reference for *BTG2* and *PPP1CA* genes (A, B), and the SW480 cell line was applied as a reference for the *PEG3* gene (C) with an expression level set to 1.0. Expressions in all other cells were presented as an n-fold difference compared with the references. Mean ± SD of three experiments is reported. (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

considered as the baseline/reference for *PEG3* expression. The HT29/219 ($p<0.01$), HCT116, and SW742 cell lines ($p<0.05$) had significantly higher *PEG3* expression levels compared with SW480. However, no such difference was observed in the SW48 and LS180 cell lines.

## The methylation status of *BTG2*, *PPP1CA*, and *PEG3* genes differ in CRC cell lines

Methylation patterns were determined by MSP (*BTG2* and *PPP1CA*) and bisulfite sequencing PCR (*PEG3*) methods before and after treatment by 5-Aza and/or TSA. As shown in Figure 2, both *BTG2* and *PPP1CA* were completely methylated in the SW48 cell line. Heterologously methylated *BTG2* was observed in HT29/219, SW748, and SW480, and the heterologously methylated *PPP1CA* was found in SW480, SW748, and LS180 cell lines. *BTG2* was unmethylated in the HCT116 and LS180 cell lines, and *PPP1CA* was unmethylated in HCT116 and HT29/219 cell lines. The results indicated that demethylation of both *BTG2* and *PPP1CA* genes was synergically increased in the presence of TSA along with 5-Aza (Figure 2). The methylation pattern of 23 CpG sites of the *PEG3* gene is presented in Figure 3. As an imprinted gene, *PEG3* is exclusively expressed from the paternal allele, and the

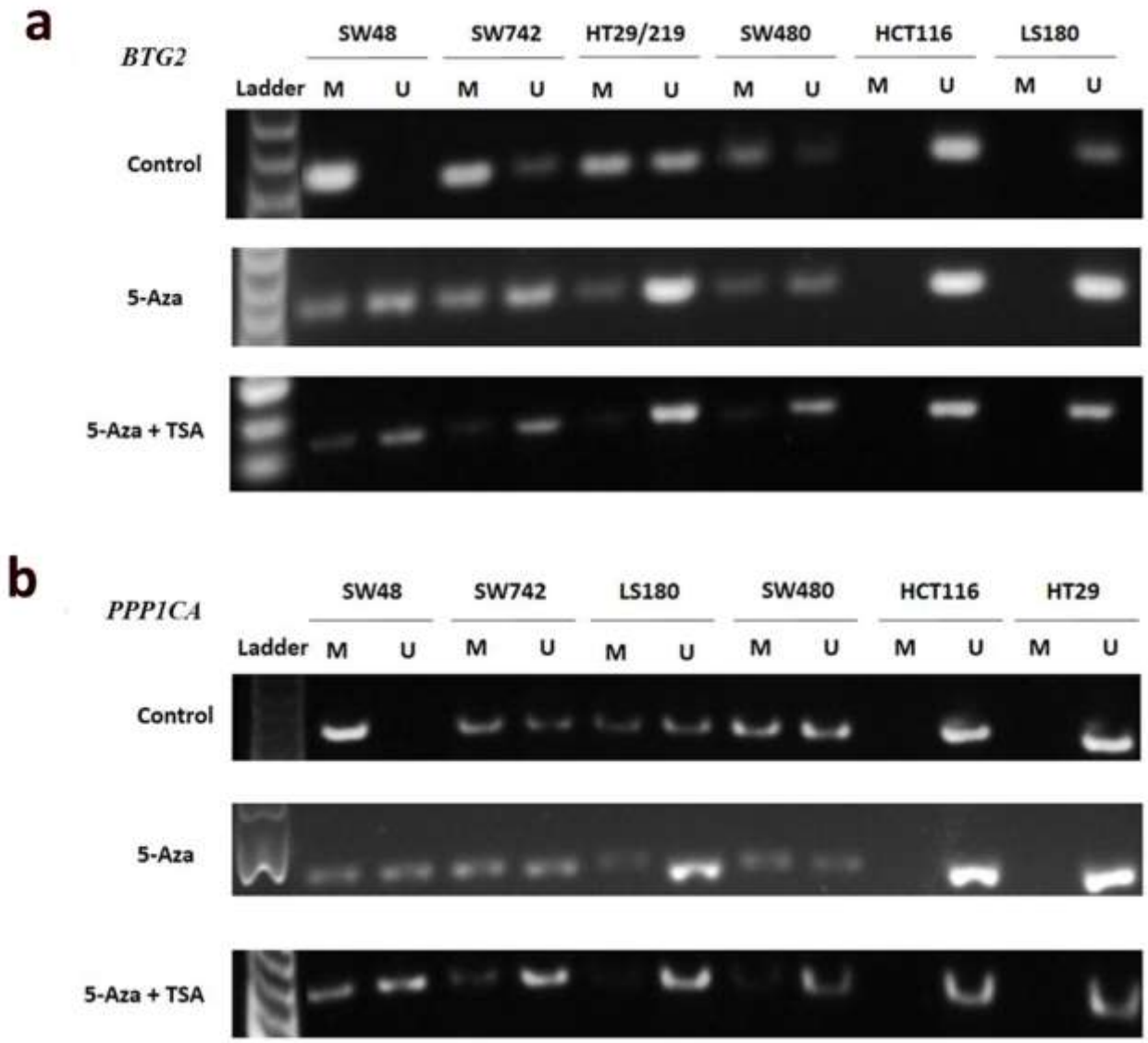


**Figure 2.** Methylation status of *BTG2* (A) and *PPP1CA* (B) genes was determined by MSP method before and after treatment with 5-Azacitidine or 5-Azacitidine + Trichostatin A in colon adenocarcinoma cell lines. U: unmethylated, M: methylated.

maternal allele is normally methylated. As illustrated in Figure 3, the highest paternal allele methylation was observed in the SW480 cell line. *PEG3* was also considerably methylated in SW742, LS180, and SW48 and low methylated in HT29/219 and HCT116. Most of the CpG sites of *PEG3* were unmethylated in normal colon tissues (Figure 3, part B). Evaluation results showed that 5-Aza demethylated most CpG sites of the *PEG3* gene in SW480, which had the highest methylated *PEG3*.

## Induction of *BTG2*, *PPP1CA*, and *PEG3* gene expression after treatment with DNA methyltransferase and histone deacetylase inhibitors in a cell line-dependent manner

To evaluate the association between DNA methylation, histone acetylation, and silencing of the *BTG2*, *PPP1CA*, and *PEG3* genes, gene expression levels were determined by real-time quantitative PCR assay after treatment by 5-Aza and/or TSA. and

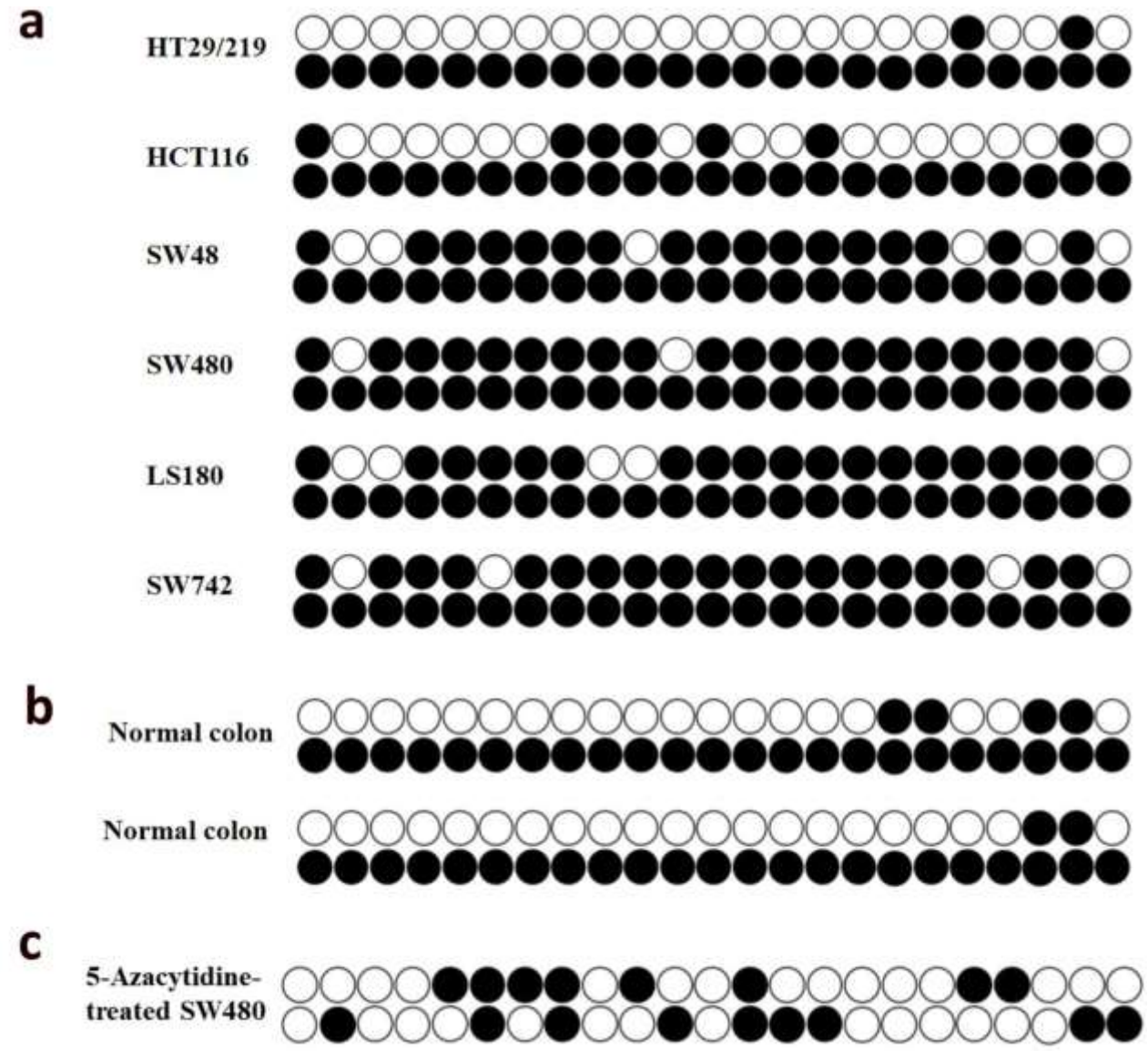


**Figure 3.** Methylation patterns of *PEG3* imprinted gene in colon adenocarcinoma cell lines were detected by bisulfite sequencing PCR. The methylation status of 23 CpG sites in the promoter was assessed using genomic DNA from six cell lines (A), normal colon tissues (B), and SW480 after treatment by 5-Azacitidine (C). Each circle indicates one CpG site. Circles filled with black represent methylated CpG sites, and open circles indicate unmethylated CpG sites.

compared between treated groups and the vehicle group (DMSO-treated cell lines). The results for the *BTG2*, *PPP1CA* and *PEG3* genes are presented in Figures 4, 5, and 6, respectively. As can be seen in these figures, treatment by 5-Aza and/or TSA significantly increased the expression level of *PEG3* but not those of *BTG2* and *PPP1CA* in the SW480 cell line. 5-Aza alone and combined with TSA significantly enhanced the expression levels of all three genes in the SW48 and SW742 cell lines; however, TSA alone had such an effect only on the *PEG3* gene in the SW48 cell line and on the *BTG2* gene in the SW742 cell line. We also found that treatment by 5-Aza alone did not increase the expression levels of all three genes in the HCT116 and HT29/219 cell lines, although TSA alone and combined with 5-Aza significantly enhanced the BTG2 and *PPP1CA* expression levels in the HT29/219 cell line and the *PEG3* expression level in the HCT116 cell line. According to the results for the LS180 cell line, TSA alone and combined with 5-Aza significantly increased the expression of all three genes; however, after treatment with 5-Aza alone, significant expression enhancement was only observed for *PEG3* in the LS180 cell line.

## Discussion

Evidence indicates that epigenetic modifications like DNA methylation and histone acetylation can lead to loss of different tumor suppressor genes expression in CRC (37). Further investigations to find other genes with aberrant epigenetic alterations in CRC can contribute to recognizing new epigenetic biomarkers for the diagnosis, prognosis, and prediction of CRC. In a continuing effort to recognize other genes involved in CRC, our aims were to evaluate the expression of *BTG2*, *PPP1CA*, and *PEG3* genes in colon adenocarcinoma cell lines and to investigate epigenetic modification as a possible mechanism for inactivation of these genes.

Previous studies have illustrated the downregulation and tumor suppressor activity of *BTG2* in various cancers including lung, breast, prostate, liver, kidney, melanoma, larynx, and gastric cancer (14, 16-22). The association between methylation and reduced *BTG2* expression in non-small cell lung cancer was reported by Shen et al. (14). This association was also observed in B-cell acute lymphoblastic leukemia (B-ALL) by et al. (32). Similarly, we evaluated the *BTG2* gene expression and methylation status in six human colon

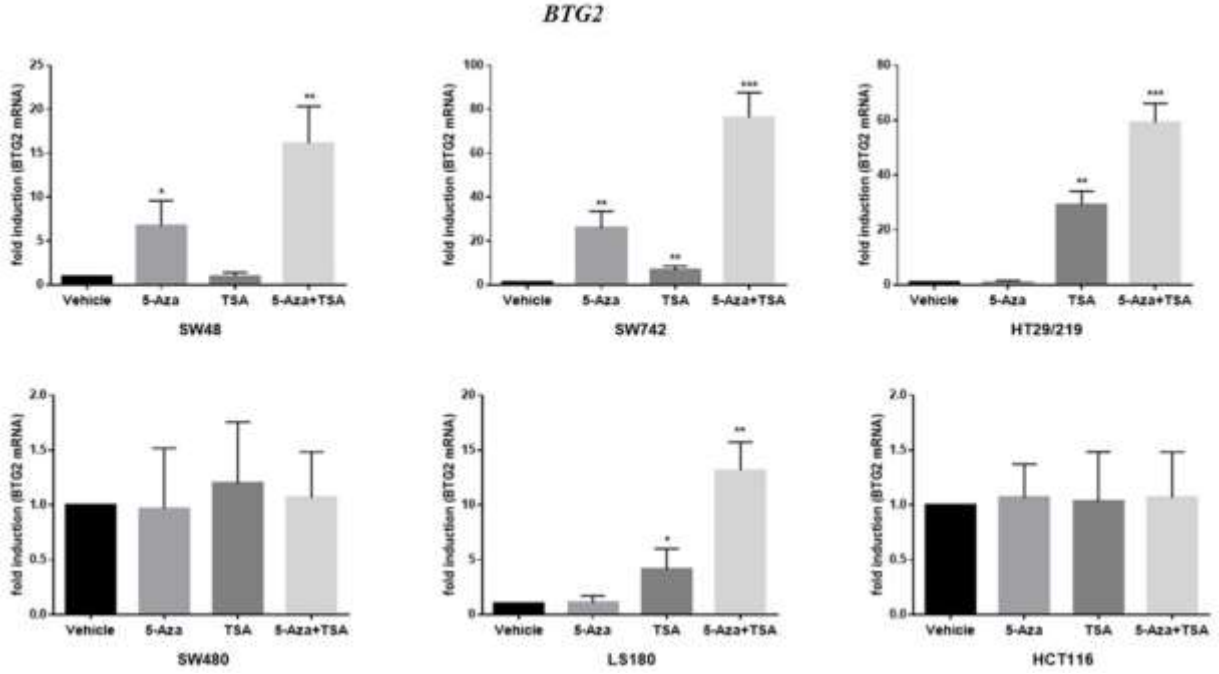


**Figure 4.** Relative *BTG2* mRNA expression in colon adenocarcinoma cell lines after 5-Azacitidine and/or Trichostatin A treatment was determined by Real-Time RT-PCR. *BTG2* mRNA expression was normalized to GAPDH mRNA. Each DMSO-treated cell line was considered as the vehicle control, with an expression level set to 1.0. *BTG2* expression levels after treatments were presented as an n-fold difference compared with the vehicle control. (*p<0.05, **p<0.01, ***p<0.001)

carcinoma cell lines. *BTG2* mRNA expression was quantitatively correlated to its promoter methylation

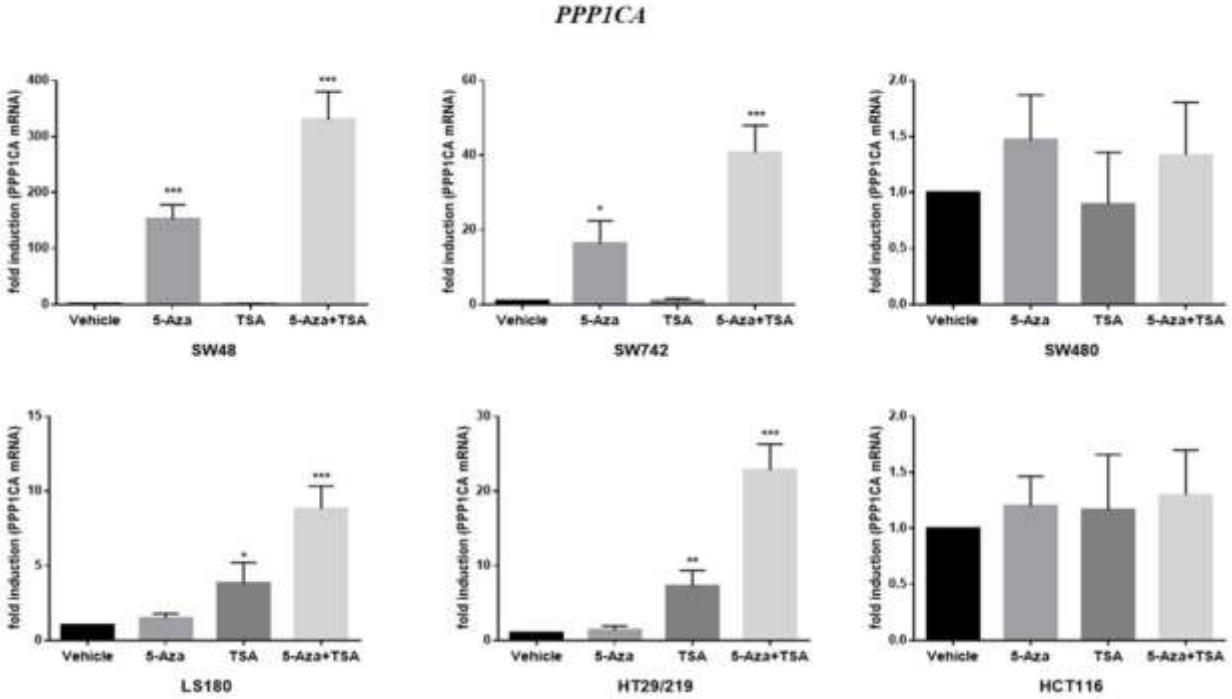


**Figure 5.** Relative *PPP1CA* mRNA expression in colon adenocarcinoma cell lines after 5-Azacitidine and/or Trichostatin A treatment was determined by Real-Time RT-PCR. *PPP1CA* mRNA expression was normalized to GAPDH mRNA control. Each DMSO-treated cell line was considered as the vehicle control, with an expression level set to 1.0. *PPP1CA* expression levels after treatments were presented as an n-fold difference compared with the vehicle control. (*p<0.05, **p<0.01, ***p<0.001)

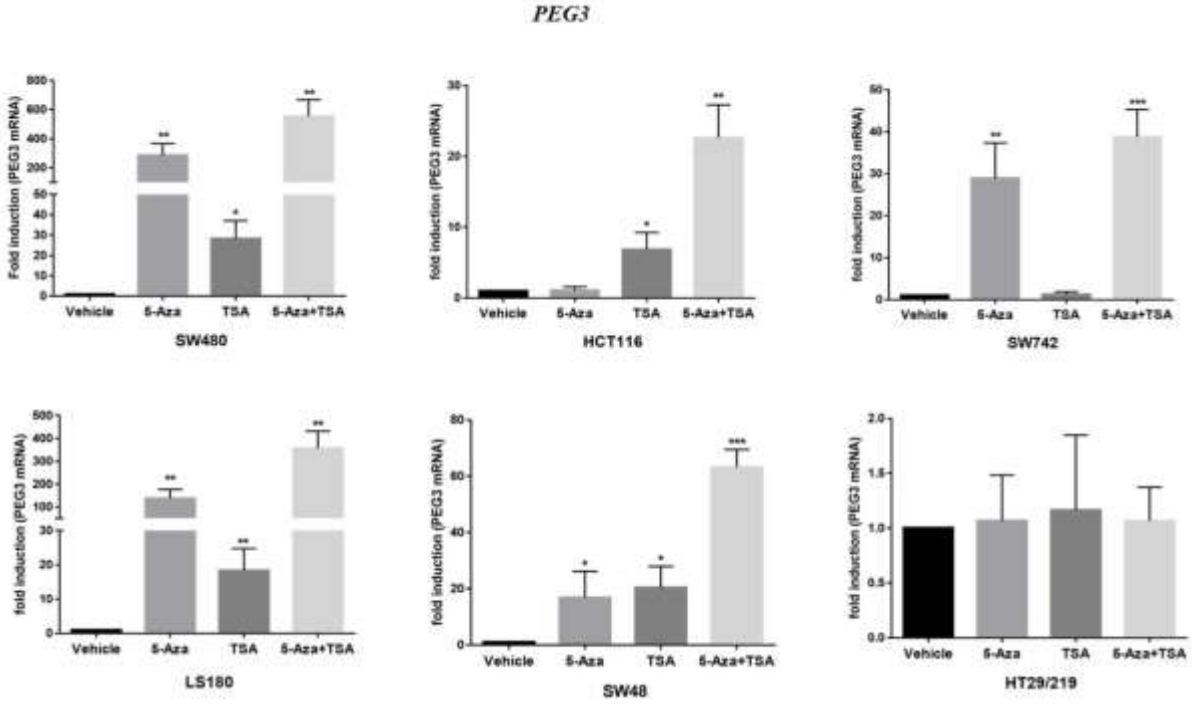


**Figure 6.** Relative *PEG3* mRNA expression in colon adenocarcinoma cell lines after 5-Azacitidine and/or Trichostatin A treatment was determined by Real-Time RT-PCR. *PEG3* mRNA expression was normalized to GAPDH mRNA. Each DMSO-treated cell line was considered as the vehicle control, with an expression level set to 1.0. *PEG3* expression levels after treatments were presented as an n-fold difference compared with the vehicle control. (*p<0.05, **p<0.01, ***p<0.001)

status. Our results indicated that the SW48 and SW480 cell lines, which were highly methylated for BTG2, virtually did not express *BTG2*. In contrast, the HCT116 cell line, displaying the *BTG2* unmethylated allele only, showed the highest expression level of *BTG2* compared to other cell lines. We further investigated whether treatment with 5-Aza as a demethylating agent and TSA as a histone deacethylase inhibitor can change *BTG2* gene expression. Our results indicated that 5-Aza and TSA, particularly their combination therapy, can increase *BTG2* expression in a cell line-dependent manner according to their methylation pattern.

The tumor suppressor role of *PPP1CA* and its repression was reported by E. Castro et al. in small intestine, prostate, vulva, kidney, and colorectal tumors (31). It has also been shown that methylation reduces *PPP1CA* expression in B-cell acute lymphoblastic leukemia (B-ALL) (32). Similarly, we found that methylation is the suppression mechanism for *PPP1CA* expression. In this regard, treatments with 5-Aza and TSA, especially in combination with each other, were effective in enhancing *PPP1CA* expression in a cell line-dependent manner.

Chen et al. introduced *PEG3* as a tumor suppressor gene in colorectal cancer (38). Zhou et al. also indicated that *PEG3* mRNA expression was significantly lower in colon cancer tissues compared with their adjacent tissue, which suggests *PEG3* as a tumor suppressor gene in the nucleus. Nonetheless, they intriguingly found that accumulation of the *PEG3* protein in the cytosol may be associated with the progression and prognosis of colon cancer (29). Previous studies found methylation-induced downregulation of *PEG3* in breast, ovary, cervix, and glioma cancers (24, 26-28).

Consistent with previous studies, the current results revealed that *PEG3* expression is significantly associated with its promoter methylation. Similar to *BTG2* and *PPP1CA* genes, *PEG3* expression was also significantly increased after 5-Aza and/or TSA treatment in a cell line-dependent manner. It has been shown that DNA methylation and histone acetylation have a synergetic cross-talk in tumorigenesis (39). In this regard, Yang et al. reported combination therapy of 5-Aza and TSA as a potential therapy for lung cancer (33). Similarly, we found that the combination of 5-Aza and TSA had the best effects on *BTG2*, *PPP1CA*, and *PEG3* in all cell lines compared with either 5-Aza or TSA alone. Hence, the combination of these drugs may be considered as a potential therapy for colon cancer. A brief working hypothesis graph is shown in Figure 7.

The current study had certain limitations: the expression of *BTG2*, *PPP1CA*, and *PEG3* genes was

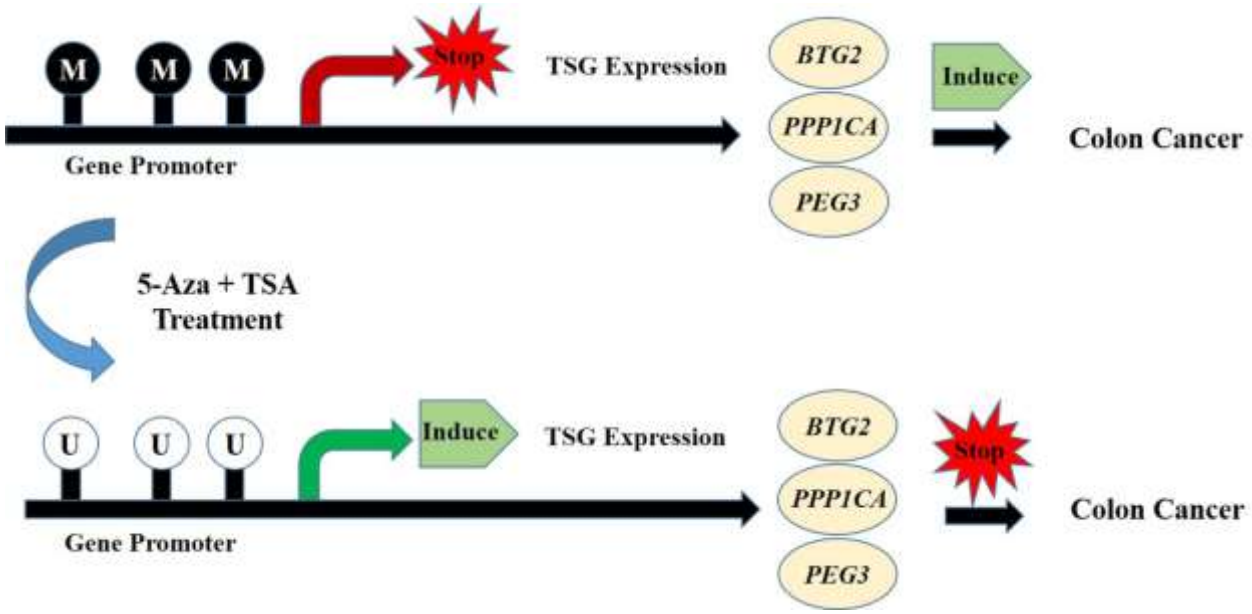


**Figure 7.** A brief working hypothesis graph of the present study. M: Methylated, U: Unmethylated, 5-Aza: 5-Azacitidine, TSA: Trichostatin A, TSG: tumor suppressor gene.

not evaluated in normal cell lines, the phenotypical alterations of the cells upon treatment with 5Aza and TSA were not assessed, and positive/negative controls were not considered.

## Conclusion

From the overall results of this study, it might be concluded that there is an inverse correlation between *BTG2*, *PPP1CA*, and *PEG3* genes expression and promoter methylation levels, suggesting that epigenetic modifications may be the mechanism behind the expression regulation of these genes in colon cancer cells. Therefore, these genes may be targeted for demethylation approaches and, consequently, colon cancer treatment. The combination of 5-Aza and TSA may be a promising therapeutic approach for colon cancer. Further investigations are required to confirm this experimental conclusion.

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## Conflict of interests

The authors declare that they have no conflict of interest.

## References

1. Mattiuzzi C, Sanchis-Gomar F, Lippi G. Concise update on colorectal cancer epidemiology. Ann Transl Med 2019;7.

2. Mokarram P, Zamani M, Kavousipour S, Naghibalhossaini F, Irajie C, Sarabi MM, et al. Different patterns of DNA methylation of the two distinct O6-methylguanine-DNA methyltransferase (O 6-MGMT) promoter regions in colorectal cancer. Mol Biol Rep 2013;40:3851-7.

3. Hosseini SV, Mojtahedi Z, Beizavi Z, Khazraei H, Zamani M. Relationship between Arg753Gln Toll-like receptor 2 and Asp299Gly Toll-like receptor 4 genetic variations and susceptibility to colorectal cancer in southern Iran. Arch Biol Sci 2018;70:775-9.

4. Zamani M, Hosseini SV, Mokarram P. Epigenetic biomarkers in colorectal cancer: premises and prospects. Biomarkers 2018;23:105-14.

5. Okugawa Y, Grady WM, Goel A. Epigenetic alterations in colorectal cancer: emerging biomarkers. Gastroenterology 2015;149:1204-25.

6. Li J, Sun Ch, Cai W, Li J, Rosen B P, Chen J. Insights into S-adenosyl-l-methionine (SAM)-dependent methyltransferase related diseases and genetic polymorphisms. Mutat Res Rev Mutat Res 2021;788:108396.

7. Baylin SB, Jones PA. Epigenetic determinants of cancer. CSH Perspect Biol 2016;8:a019505.

8. Aslani S, Mahmoudi M, Karami J, Jamshidi AR, Malekshahi Z, Nicknam MH. Epigenetic alterations underlying autoimmune diseases. Autoimmunity 2016;49:69-83.

9. Wise IA, Charchar FJ. Epigenetic modifications in essential hypertension. Int J Mol Sci 2016;17:451.

10. Khyzha N, Alizada A, Wilson MD, Fish JE. Epigenetics of atherosclerosis: emerging mechanisms and methods. Trends Mol Med 2017;23:332-47.

11. Ordovás JM, Smith CE. Epigenetics and cardiovascular disease. Nat Rev Cardiol 2010;7:510.

12. Abdolmaleky HM, Thiagalingam S, Wilcox M. Genetics and epigenetics in major psychiatric disorders. Am J Pharmacogenomics 2005;5:149-60.

13. Drong A, Lindgren C, McCarthy M. The genetic and epigenetic basis of type 2 diabetes and obesity. Clin Pharmacol Ther 2012;92:707-15.

14. Shen S, Zhang R, Guo Y, Loehrer E, Wei Y, Zhu Y, et al. A multi-omic study reveals *BTG2* as a reliable prognostic marker for early-stage non-small cell lung cancer. Mol Oncol 2018;12:913-24.

15. Zhou L, Liang X, Zhang L, Yang L, Nagao N, Wu H, et al. MiR-27a-3p functions as an oncogene in gastric cancer by targeting BTG2. Oncotarget 2016;7:51943.

16. Zhang Y-j, Wei L, Liu M, Li J, Zheng Y-q, Gao Y, et al. *BTG2* inhibits the proliferation, invasion, and apoptosis of MDA-MB-231 triple-negative breast cancer cells. Tumor Biol 2013;34:1605-13.

17. Coppola V, Musumeci M, Patrizii M, Cannistraci A, Addario A, Maugeri-Sacca M, et al. *BTG2* loss and miR-21 upregulation contribute to prostate cell transformation by inducing luminal markers expression and epithelial–mesenchymal transition. Oncogene 2013;32:1843-53.

18. Huang C-S, Zhai J-M, Zhu X-X, Cai J-P, Chen W, Li J-H, et al. *BTG2* is Down-regulated and inhibits Cancer stem cell-like features of side population cells in hepatocellular carcinoma. Digest Dis Sci 2017;62:3501-10.

19. Struckmann K, Schraml P, Simon R, Elmenhorst K, Mirlacher M, Kononen J, et al. Impaired expression of the cell cycle regulator BTG2 is common in clear cell renal cell carcinoma. Cancer Res 2004;64:1632-8.

20. Yang CH, Yue J, Pfeffer SR, Handorf CR, Pfeffer LM. MicroRNA miR-21 regulates the metastatic behavior of B16 melanoma cells. J Biol Chem 2011;286:39172-8.

21. Liu M, Wu H, Liu T, Li Y, Wang F, Wan H, et al. Regulation of the cell cycle gene, *BTG2*, by miR-21 in human laryngeal carcinoma. Cell Res 2009;19:828-37.

22. Zhang L, Huang H, Wu K, Wang M, Wu B. Impact of *BTG2* expression on proliferation and invasion of gastric cancer cells in vitro. Mol Biol Rep 2010;37:2579-86.

23. Li B, Liu X, Wu G, Liu J, Cai S, Wang F, et al. MicroRNA-934 facilitates cell proliferation, migration, invasion and angiogenesis in colorectal cancer by targeting B-cell translocation gene 2: Running title: miR934 facilitates the progression of colorectal cancer. Bioengineered 2021;12: 9507-19.

24. Feng W, Marquez RT, Lu Z, Liu J, Lu KH, Issa JPJ, et al. Imprinted tumor suppressor genes *ARHI* and *PEG3* are the most

frequently down-regulated in human ovarian cancers by loss of heterozygosity and promoter methylation. Cancer 2008;112:1489-502.

25. Kim J, Frey WD, He H, Kim H, Ekram MB, Bakshi A, et al. *PEG3* mutational effects on reproduction and placenta-specific gene families. PloS one 2013;8:83359.

26. Dowdy SC, Gostout BS, Shridhar V, Wu X, Smith DI, Podratz KC, et al. Biallelic methylation and silencing of paternally expressed gene 3 (*PEG3*) in gynecologic cancer cell lines. Gynecol Oncol 2005;99:126-34.

27. Chen MY, Liao WSL, Lu Z, Bornmann WG, Hennessey V, Washington MN, et al. Decitabine and suberoylanilide hydroxamic acid (SAHA) inhibit growth of ovarian cancer cell lines and xenografts while inducing expression of imprinted tumor suppressor genes, apoptosis, G2/M arrest, and autophagy. Cancer 2011;117:4424-38.

28. Maegawa S, Yoshioka H, Itaba N, Kubota N, Nishihara S, Shirayoshi Y, et al. Epigenetic silencing of *PEG3* gene expression in human glioma cell lines. Mol Carcinog 2001;31:1-9.

29. Zhou T, Lin W, Zhu Q, Renaud H, Liu X, Li R, et al. The role of *PEG3* in the occurrence and prognosis of colon cancer. OncoTargets Ther 2019;12:6001.

30. Aggen JB, Nairn AC, Chamberlin R. Regulation of protein phosphatase-1. Chem Biol 2000;7:13-23.

31. Castro ME, Ferrer I, Cascón A, Guijarro MV, Lleonart M, Cajal SRy, et al. *PPP1CA* contributes to the senescence program induced by oncogenic Ras. Carcinogenesis 2008;29:491-9.

32. Vafadar A, Mokaram P, Erfani M, Yousefi Z, Farhadi A, Elham Shirazi T, et al. The effect of decitabine on the expression and methylation of the *PPP1CA*, *BTG2*, and *PTEN* in association with changes in miR-125b, miR-17, and miR-181b in NALM6 cell line. J Cell Biochem 2019;120:13156-67.

33. Yang Y, Yin W, Wu F, Fan J. Combination of azacitidine and trichostatin A decreased the tumorigenic potential of lung cancer cells. OncoTargets Ther 2017;10:2993.

34. Capobianco E, Mora A, La Sala D, Roberti A, Zaki N, Badidi E, et al. Separate and combined effects of DNMT and HDAC inhibitors in treating human multi-drug resistant osteosarcoma HosDXR150 cell line. PloS one 2014;9:95596.

35. Al-Rayyan N, Litchfield LM, Ivanova MM, Radde BN, Cheng A, Elbedewy A, et al. 5-Aza-2-deoxycytidine and trichostatin A increase COUP-TFII expression in antiestrogen-resistant breast cancer cell lines. Cancer Lett 2014;347:139-50.

36. Erfani M, Hosseini SV, Mokhtari M, Zamani M, Tahmasebi K, Alizadeh Naini M, et al. Altered ARID1A expression in colorectal cancer. BMC cancer 2020;20:1-13.

37. Vymetalkova V, Vodicka P, Vodenkova S, Alonso S, Schneider-Stock R. DNA methylation and chromatin modifiers in colorectal cancer. Mol Aspects Med 2019;69:73-92.

38. Chen G, Han N, Li G, Li X, Li G, Li Z, et al. Time course analysis based on gene expression profile and identification of target molecules for colorectal cancer. Cancer Cell Int 2016;16:22.

39. Ou J-N, Torrisani J, Unterberger A, Provençal N, Shikimi K, Karimi M, et al. Histone deacetylase inhibitor Trichostatin A induces global and gene-specific DNA demethylation in human cancer cell lines. Biochem Pharmacol 2007;73:1297-307.