Chapter 8

The Future of Ovarian Cancer Treatment: The Promise of Epigenetic Markers

Fatimeh Alghazawi[1]

Nastaran Azami[2]

Amirkasra Dehghani[3]

Elif Sibel Aslan[4]

Sajjad Eslamkhah[5]

Abstract

Epigenetics refers to inheritable DNA changes that occur without altering the DNA sequence itself. It encompasses processes like DNA methylation, histone modifications, and non-coding regulatory RNA pathways, all of which influence gene activity in mammals. The Ten-Eleven Translocate (TET) enzyme family, including TET1, TET2, and TET3, plays a role in regulating DNA methylation and gene expression. TET1 specifically converts methylated cytosine to 5-hydroxymethylcytosine (5hmC) and protects CpG islands (CGIs) from improper methylation. In ovarian cancer, TET2 gene mutations have been associated with higher tumor grade, advanced stage, lymph node metastases, and vascular thrombosis. TET2 acts as a tumor suppressor gene, and its suppression may contribute to disease development. TET3, inherited from oocytes, is linked to several diseases, including ovarian cancer. Increased TET3 expression in ovarian cancer is associated with poor outcomes and prognosis, making it a potential indicator for the disease. While vitamin C's effectiveness against ovarian cancer is still being studied, it is important to note that research is in the early stages, and further evidence

1 Department of Molecular Biology and Genetics, Faculty of Engineering and Natural Sciences, Biruni University, Istanbul, Türkiye. ORCID ID: 0009-0007-8706-2034 221106900@st.biruni.edu.tr

2 Biruni University, Istanbul, Türkiye, 0009-0006-4659-2584, 221106901@st.biruni.edu.tr

3 Biruni University, Istanbul, Türkiye, 0009-0006-3962-9908, 221106001@st.biruni.edu.tr

4 Biruni University, Istanbul, Türkiye, 0000-0002-3081-9004, easlan@biruni.edu.tr

5 0000-0001-8317-9293, sajjadeslamkhah1990@gmail.com

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is needed to establish its efficacy. Overall, epigenetic mechanisms, TET enzymes, and their mutations play crucial roles in ovarian cancer progression, providing potential targets for future therapeutic interventions.

Introduction:

Epigenetics is the study of the causal interactions between genes and their products, which create phenotypes. Humans share 99% of the same genes, making epigenetics a valuable reference for researchers and medical doctors studying diseases and disorders. C.H. Waddington's definition highlights the importance of understanding the beginnings of diseases and disorders[1]. Epigenetics involves gene expression changes without altering genetic sequence, predicting variation among humans through multiple pathways essential for maintaining tissue-specific gene expression patterns.[1]. DNA methylation and histone marks are direct mechanisms, while ncRNA is indirect. These heritable alterations are created during differentiation and preserved over cell division cycles, allowing diverse identities while maintaining genetic makeup[2]. The location of nucleosomes along the DNA, the methylation of cytosine bases in DNA, and posttranslational modifications to histone proteins are just a few examples of the epigenetic changes that are responsible for the heritability of gene expression patterns [1]. As known, a lot of diseases like diabetes, autoimmune diseases, and cancer could be affected by external factors such as aging, lifestyle, and even our prenatal development. With over 50% of new cases being identified beyond age 65 and over 70% of cancer fatalities happening in this same age group, it is well known that cancer is a disease of older individuals [3]. Ovarian cancer, the seventh most prevalent cancer worldwide, affects 4% of new cases and ranks as the ninth most common cancer-related death [3] . In the upcoming years, this ratio is expected to rise as our population ages and life expectancy rises. As a patient gets older, the results get worse and worse [3]. Epigenetics investigates the link between cancer, DNA methylation, and demethylation, in addition to how these factors influence cancer progression and chemo-resistance, particularly ovarian cancer[4]. Researchers explore using intravenous ascorbic acid (AA) for cancer treatment, with high doses recommended for OV [5]. In this review, the impact of one of the latest discovered enzymes are Ten-Eleven Translocation enzymes OV and overdoses of Vitamin C effectiveness will be reviewed.

DNA Methylation

The fundamental concept of DNA methylation is crucial to understanding the mechanism of TET enzymes. DNA methylation involves the addition of

methyl groups to the DNA molecule, which contributes to its preservation and stability [6]. This process can modulate the activity of a DNA sequence while preserving its sequence integrity [6, 7, 8, 9]. Methylation serves as an important inherited epigenetic mark. DNA methyltransferases enzymes (DNMTs), which are highly conserved among mammals, are responsible for transferring methyl groups to the DNA strand. DNMT1, DNMT3A, and DNMT3B are enzymatically active DNMTs that play key roles in initiating and maintaining DNA methylation, an extensively studied epigenetic modification in mammals [1]. In mice, the deletion of DNMT1 leads to embryonic mortality and a significant reduction in global DNA methylation levels. DNMT1 is the primary enzyme responsible for maintaining DNA methylation patterns following DNA replication. It exhibits a preference for methylating hemi methylated DNA templates, where only one DNA strand is methylated [10]. During the S phase of the cell cycle, DNMT1 is localized at DNA replication forks, indicating its role in maintenance activities. Recent studies have revealed that the accessory protein UHRF1 plays a crucial role in facilitating the proper targeting of DNMT1 to replicate DNA [11]. UHRF1 achieves this through its specific SRA domain, which enables its binding to hemi methylated DNA. An examination of DNMT1-knockout mouse embryonic DNA revealed the presence of de novo DNA methyltransferase (DNMT) activity[10]. DNMT1 primarily targets CpG regions (regions rich in cytosine-guanine dinucleotides) for DNA methylation, with the "p" representing the phosphate group [10]. DNMT3A and DNMT3B are classified as de novo methyltransferases. They play a crucial role in depositing initial methylation marks on unmethylated CpG islands during early embryogenesis and in primordial germ cells [12]. DNMT3L serves as the homolog of DNMT3A and DNMT3B, and its presence increases the number of de novo methyltransferases available for methyl group donation from S-adenosyl-l-methionine (SAM) [12]. In the human genome, approximately 70-80% of CpG regions are methylated [13]. After adding a methyl group to the base (in both strands), it's a mark to not express this gene segment which means inactivation. However, DNA methylation controls DNA replication as well by inactivation of many replication suppressors and promoters which means gene expression ([6]. Elevated methylation of CpG-rich regions is a common occurrence in various tumors, including ovarian tumors. However, it is important to note that not all CpG-rich regions associated with tumors are gene promoters [14]. Aberrant methylation of CpG islands in ovarian tumors has been linked to the repression of genes involved in crucial processes such as cell cycle control, apoptosis, drug sensitivity, and tumor suppression [15].

Ten-Eleven Translocation enzymes

Ten-eleven translocation enzymes (TET) family of dioxygenases plays an important role in the process of regulating transcription and DNA demethylation. Noteworthy that those enzymes only exist in mammalians [16]. TET1, TET2, and TET3 are large proteins and have multidomain functional sides. At 10q21.3 where TET1 located, TET2 is found on chromosome 4q24, and TET3 is found on chromosome 2p13.1 [17]. When a lack of methylation emerges in the newly produced DNA strand during replication, which may be caused by the presence of 5-hmC in the parental strand, TET enzymes are implicated in the passive DNA demethylation that takes place [17]. TET proteins share common structural features, including a conserved C-terminal region (cys-rich region) and a double-stranded β-helix (DSBH) domain. One important DNA base modification is 5-methylcytosine, which arises from the methylation of cytosine (C) and frequently plays a role in regulating gene transcription and other genomic functions [9, 18]. TET1 and TET3 contain a Cysteine-X-X-Cysteine (CXXC) domain that enables them to interact with both methylated and unmethylated CpG regions on DNA [19]. However, TET2 does not possess the CXXC domain. Instead, it forms a partnership with an independent protein called IDAX, which contains the CXXC domain [19]. As a result, the TET enzymes stop DNA's 5-methylcytosine (5mC) from being hydroxylated into 5-hydroxymethylcytosine (5hmC), and they subsequently catalyze the oxidation of 5hmC into 5-formylcytosine (5fC), and finally into 5-carboxycytosine (5caC) (Figure 1). Mutations in TET proteins and dysregulated regulation of their activity are implicated in the development of various human diseases, including cancer [13].

Antigen and cytokine receptors continuously send signals to T and B cells during immunological responses and development. These outside signals come together and are deciphered by combining transcription factors that are both widely expressed and unique to certain cell types, which work with chromatin regulators to alter the epigenome [20]. DNA and histone modifications, which enable information to be stored and/or passed down to daughter cells, are among the epigenetic changes connected to immune cell activation and differentiation [21]. As shown above, studies of the distribution of 5hmC over the entire genome show a strong connection between 5hmC and gene transcription. The quantity of 5hmC at gene bodies in thymic and peripheral T cell subsets correlates strongly positively with gene expression, RNA polymerase II occupancy, and H3K36me3 levels [21].

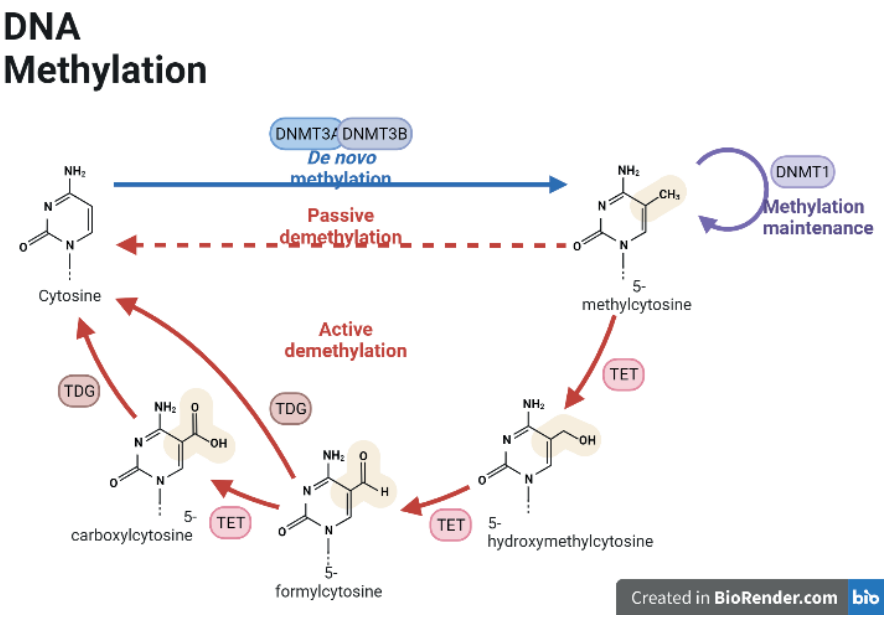

***Figure 1** depicts the fundamental concept of DNA methylation/demethylation and the involvement of Ten-Eleven Translocation (TET) proteins. TET proteins play a vital role in catalyzing a series of sequential oxidations of 5-methylcytosine (5-mC) to generate 5-hydroxymethylcytosine (5-hmC), 5-formylcytosine (5-fC), and 5-carboxylcytosine (5-caC). Additionally, TET proteins can directly convert 5-mC to 5-fC and 5-caC [13].*

TET1 role on Ovarian Cancer

TET1's role in cancer has been extensively studied and has generated significant interest in scientific investigations. Studies have revealed that TET1 plays a crucial role in regulating DNA methylation and gene expression by catalyzing the conversion of methylated cytosine to 5-hydroxymethylcytosine (5hmC) [22]. Additionally, TET1 possesses a CXXC domain that binds to unmethylated CpG islands, thereby safeguarding them from abnormal methylation. TET1 functions as a tumor suppressor and has been found to inhibit the migration and invasion of papillary thyroid cancer cells. Recent research has also highlighted its significant impact on ovarian cancer cells [22].

Studies conducted in vivo and in vitro have revealed that TET1 elevated 5-hmC levels in ovarian cancer, which in turn prevented colony formation and cell proliferation by expressing RASSF5, a tumor suppressor gene [12]. TET1's function in preserving genomic integrity and avoiding cancer is critical. TET1 deficiency, it turns out, not only causes genomic instability,

but it can also promote tumor vascular invasion [23]. TET1 overexpression, on the other hand, it has been proven to reverse the epithelial-mesenchymal transition and prevent cancer cell metastasis [24]. Chemo-resistance is caused by deregulation of DNA methylation/demethylation, and the TET family of dioxygenases (TET1/2/3) plays an important role in this. TET1 was significantly upregulated in cisplatin-resistant CP70 cells, and increased re-expression of vimentin caused partial epithelial-to-mesenchymal (EMT) [25]. TET1 was detected by immunohistochemistry in the nucleus and cytoplasm of human ovarian cancer tissues, and it was found to be positively linked with the presence of residual tumor and chemotherapy response [25].

TET2 and Ovarian Cancer

Thousands of women throughout the world are affected by the extremely aggressive and challenging-to-treat disease known as ovarian cancer. The TET2 enzyme may have a substantial influence on ovarian cancer, according to a recent study, opening new treatment and management options. Studies have shown that 5-hmC and TET2 expression are much lower in epithelial ovarian cancer than in healthy ovarian tissues [16]. Furthermore, in other studies, it was discovered that low TET2 and 5-hmC levels were linked to high tumor grade, pathologic stage, lymph node metastases, and vascular thrombosis in 130 people with epithelial ovarian cancer ([12]. TET2 and 5-hmC levels were significantly linked with poor clinical outcomes, such as a shorter time to the first recurrence, in high-grade and serous ovarian cancer [17][12]. TET2 mutations are seen in both germline and somatic ovarian cancer patients. TET2 may function as a tumor suppressor gene in ovarian cancer, and its dysregulation may help in the disease's development, according to these observations [18].In addition, studies have shown that TET2 suppression might encourage the development and invasion of ovarian cancer cells. On the other hand, new research has emphasized TET2's potential as a target for cutting-edge treatment strategies. For instance, it has been discovered that various medications, including VC, Metformin, and 5-Aza-2'-deoxycytidine, improve TET2 stability and may be utilized to prevent the growth of ovarian cancer [19]. Additionally , the discovery of germline TET2 variations in ovarian cancer patients raises the possibility that genetic testing for such variants might enhance risk assessment and direct patients' individualized treatment regimens. Overall, greater research into TET2's effects on ovarian cancer is necessary since it may have effects on diagnosis, prognosis, and therapy [20]. Another article presents our current knowledge of TET2's relationship to ovarian cancer. It provides an overview of the available studies that points to a potential involvement for

TET2 in the emergence and spread of ovarian cancer. The article also covers potential directions for novel therapies and management tactics that aim to reduce TET2 expression or stability [21].

TET3 impact on Cancer

TET3 exhibits the highest expression levels among the TET enzymes in oocytes and fertilized zygotes, while TET1 and TET2 become more predominant during early development [22]. In mouse genetic studies, TET3, inherited exclusively from oocytes, has been identified as the driver of 5mC loss and 5hmC gain in the paternal genome [23]. In addition to its association with various diseases, TET3 has been linked to several other disorders. Loss-of-function alleles of TET3 show high sensitivity in human control databases, and homozygous missense variations in TET3 have recently been connected to autosomal-recessive intellectual impairment in a consanguineous family (referred to as family 3) [9]. TET3 plays a critical role in actively reversing DNA methylation during development. Individuals with TET3 deficiency and other Mendelian disorders related to the epigenetic machinery commonly exhibit phenotypic characteristics such as developmental delay, intellectual disability, neurobehavioral symptoms, and growth anomalies [9]. TET3 has been described as an oncogene or tumor suppressor in ovarian cancer during carcinogenesis [24]. TET3 has been implicated in the inhibition of epithelial-mesenchymal transition (EMT) induced by TGF-β1, thereby exerting a protective effect against ovarian cancer [25]. Previous research has demonstrated the overexpression of TET3 in ovarian cancer tissue, although its prognostic significance and clinicopathological roles remain unclear. To elucidate the function of TET3, researchers conducted an integrated investigation using bioinformatics analysis [24]. The abundance of TET3 protein in oocytes and fertilized zygotes was confirmed [24]. TET3 expression in ovarian cancer was evaluated using the Oncomine database, as well as the TCGA and GTEx databases [14]. The relationship between TET3 gene alterations and clinicopathological features was examined through an integrative analysis of GEO datasets. Copy number alteration (CNA) and mutation analyses using cBioPortal revealed TET3 gains and diploid status, but not deletions, in ovarian cancer [14]. Furthermore, high levels of TET3 were associated with poor survival in ovarian cancer patients, as determined by the Kaplan-Meier plotter (K-M plotter) analysis [14]. This association was further validated through examination of the PrognoScan database and gene differential analyses using TCGA and GTEx data [14]. This study is the first to establish a correlation between increased TET3 expression, adverse clinicopathological outcomes, and a poor prognosis, suggesting that TET3

may serve as a diagnostic marker or therapeutic target for ovarian cancer due to its involvement in epigenetic modifications and methylation changes [14].

The Role of Vitamin C:

Numerous studies have indicated the potential of vitamin C as a treatment for ovarian cancer, as it exhibits inhibitory effects on the growth and metastasis of ovarian cancer cells. The field of cancer immunotherapy has recently garnered significant attention, leading to speculation about the potential of vitamin C supplementation to enhance immune responses and induce antitumor activity in cancer patients [26]. Moreover, clinical trials involving high-dose intravenous vitamin C administration to cancer patients have demonstrated improvements in various aspects of quality of life, including physical, mental, and emotional functions. These trials have also reported a reduction in the frequency of adverse effects such as fatigue, nausea, vomiting, and appetite loss [27].

In animal models, the co-administration of parenteral ascorbate (vitamin C) with conventional chemotherapeutic drugs carboplatin and paclitaxel has shown promising results in suppressing ovarian cancer. Furthermore, this combination therapy has demonstrated a reduction in chemotherapy-associated toxicity in ovarian cancer patients [28]. The presence of vitamin C at the reaction site is crucial for its effects. Vitamin C activates TET enzymes by reducing the embedded iron ion within TET's catalytic site. This reduction of Fe+3 to active Fe+2 restores the enzymatic activity. Interestingly, substituting vitamin C with another electron donor does not lead to a catalyzed reaction, highlighting the unique role of vitamin C in this process [29]. Vitamin C has been identified as a crucial component in the TET-mediated demethylation process, as its addition has been found to enhance TET activity [30]. Consequently, TET3 functions as a tumor suppressor. A schematic representation illustrates how vitamin C influences the TET-dependent removal of methyl groups from genomic DNA. Nevertheless, further research is required to fully comprehend the efficacy of vitamin C in ovarian cancer treatment, and it should not be used as a substitute for conventional medical interventions such as surgery, chemotherapy, and radiation [29]. Recent studies conducted on embryonic stem cells have revealed that ascorbic acid (AA), a form of vitamin C, acts as a cofactor for TET enzymes and increases their activity. In vitro experiments involving diffuse large B-cell lymphoma (DLBCL) and PTCL cells treated with intravenous AA doses demonstrated enhanced TET activity, resulting in DNA demethylation, increased expression of SMAD1 (a tumor suppressor gene), and heightened chemosensitivity of lymphoma cells [31]. Clinical

studies combining intravenous AA with chemotherapy are necessary to investigate whether AA deficiency may impact TET function and contribute to resistance in certain patients [31]. It is important to note that high doses of vitamin C can have side effects, so individuals should consult their doctor before taking large doses of vitamin C or any other supplement [30].

Conclusion:

The traditional understanding of the genetic code as the primary driver of cellular gene function and genetic changes as the main cause of human diseases has been challenged by the emergence of the epigenetic revolution in biology. In this context, TET enzymes have emerged as key players in physiology, disease, and development. The DNA demethylases belonging to the TET family have a crucial role in shaping the epigenetic landscape of tumors. While much research has focused on the direct impact of TET activity on cancer cells, it is now evident that TET involvement in the tumor microenvironment is equally critical for tumor growth and development. Recent studies have highlighted the significant role of TET1 in ovarian cancer, specifically in regulating DNA methylation patterns and gene expression. The CXXC domain of TET1 plays a protective role in preventing erroneous methylation of CGIs (CpG islands), which are important regulatory regions in the genome [32]. TET1 has an important role in maintaining genomic integrity and preventing cancer, with deficiency producing genomic instability and overexpression limiting metastasis. Recent studies suggest that the TET2 enzyme may significantly influence ovarian cancer, with studies showing lower 5-hmC and TET2 expression in epithelial ovarian cancer. These low levels are linked to high tumor grade, pathologic stage, lymph node metastases, and vascular thrombosis. TET2 mutations are present in both germline and somatic ovarian cancer patients, and their dysregulation may contribute to the disease's development. Further research is needed to understand TET2's impact on diagnosis, prognosis, and therapy. TET3 importance lies in the fact that it is the only inherited enzyme from the family of TET enzymes. Despite the lack of studies on TET3 and its relationship to ovarian cancer, the study mentioned above recommends using TET3 as a marker for ovarian cancer and, therefore, the TET3-related treatment plan. One of the most recent recommendations for cancer treatment is vitamin C. Its effect on ovarian cancer was investigated because it activates the TET enzyme, reducing cell activity and acting as a tumor suppressor. Ascorbic acid may be a potential treatment for ovarian cancer, potentially inhibiting cell growth and spreading. Cancer immunotherapy studies show potential for vitamin C supplementation to enhance immune responses in cancer patients. More research is needed to fully understand its effectiveness.

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