TET enzymes and key signalling pathways: Crosstalk in embryonic development and cancer

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Abstract

TET enzymes are responsible for catalyzing the conversion of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), during the process of active DNA demethylation. These enzymes are differentially expressed in several tissues during development and can regulate several conserved signaling pathways, such as Wingless (WNT), Notch, Sonic Hedgehog (SHH) and Transforming Growth Factor Beta (TGF-β). Low expression of *TET* genes and the consequent reduction of 5hmC levels have been commonly reported in tumors of different origins and, in most cases, associated with poor prognosis. On this basis, we aimed to compile information about the canonical action of TET enzymes on the above signaling pathways during development, as well as the alterations characterized in different cancer cells. The presence of TETs is fundamental for normal embryonic development and their deletion in animal models has shown to delay cell differentiation and result in dysregulated expression of genes involved in signaling pathways. Consequently, the absence of TETs results in central nervous system defects and retinal deformity. In cancer, low expression of TETs induces activation of the WNT, TGF-β and NOTCH pathways, either directly or indirectly. Depletion in Tet activity inhibits tumorigenic processes, such as cell proliferation and epithelial-mesenchymal transition (EMT). The prospect of TET pharmacological or molecular manipulation might have global effects that should be considered for future therapeutic intervention.

Abbreviations: TET: Ten-Eleven Translocation (Human), Tet: Ten-eleven translocation (mouse), 5mC: 5-mehtylcytosine, 5hmC: 5-hydroxymethylcytosine, WNT: Wingless, SHH: Sonic Hedgehog, TGF-β: Transforming Growth Factor Beta, EMT: epithelial-mesenchymal transition, DSHB: double-stranded β-helix domain, IDAX: Dvl-binding protein, TDG: thymine DNA glycosylase, BER: base excision repair, H3K4me3: Tri methylation of lysine 4 in the histone 3, CNS: central nervous system, DC: destruction complex, APC: Apoptosis Poliposis Coli, GSK3β: Glycogen synthase kinase-3 Beta, CK1α/γ: casein kinase alfa/gama, CKα/o: casein kinase alfa/omega, FZD: Frizzled, LRP5/6: lipoprotein receptor-related protein5/6, TCF/LEF: T-cell factor/lymphoid enhancer factor, CRC: colorectal cancer, CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats), C-myc: cancer – Myelocytomatosis, Sfrp: secreted frizzled-related protein , DKK: Dickkopf-related protein, HH: Hedgehog, IHH: Indian Hedgehog, DHH: Desert Hedgehog, PTCH1: Patched-1, SMO: Smothened, GLI: glioma-associated oncogene, SUFU: suppressor of Fused, BCL2: B-cell lymphoma 2, ANG1/2: angiopoietin, SNAIL: Zinc finger protein SNAI1, Sox2: SRY-box 2, ChIP: chromatin immunoprecipitation, Hhip: hedgehog interacting protein, Pax1/9: Paired box 1, Ccnd2: Cyclin D2, BMPs: bone morphogenetic proteins, GDF: growth and differentiation factors, SMAD: SMAD family member, FOXH1: Forkhead box protein H1, MPKs: mitogen-activated protein kinase, CDKs: cyclin dependent kinases, Smurf: SMAD ubiquitination regulatory factor, DNMT: DNA methyltransferase, PEN2: presenilin enhancer 2, APH1: Homolog B, Gamma-Secretase Subunit, NICD: Notch intracellular domain, RBP-J: recombination signal sequence-binding protein J, Hes-1: Hes Family BHLH Transcription Factor 1, Jag (1,2): Jagged DLL (1/3): Delta-like, HES5: Hes Family BHLH Transcription Factor 5, DNER: Delta/Notch Like EGF Repeat Containing, GFAP: Glial Fibrillary Acidic Protein, AXL1: AXL receptor tyrosine kinase.

Introduction

Hydroxymethylation is an epigenetic mechanism with a crucial role during development of mammals. 5-hydroxymethylcytosine (5hmC) influences chromatin structure and genomic function [1]. High levels of 5hmC are present in regulatory regions and along expressed genes and are associated with the recruitment of the transcriptional machinery. Conversion of 5mC to 5hmC is catalyzed by TET (Ten Eleven Translocation) enzymes: TET1, TET2 and TET3, which constitute a family of 2-oxoglutarate-dependent dioxygenase and iron (II). These proteins display a catalytic region in their C-terminal, with methylcytosine dioxygenase activity, composed by a conserved cysteine (*Cys-rich*) rich domain; and a double-stranded β-helix domain (DSHB), which interacts with 2-oxoglutarate and iron (II). At the N-terminal region, TET1 and TET3 show a CXXC domain, which binds to unmodified cytosines [1]. Conversely, as a consequence of a

chromosome inversion during evolution, this domain is not present in TET2; the CXXC was separated and gave origin to the *IDAX* gene, which acts as its negative regulator (Figure 1) [2].

During the process of active DNA demethylation, TET enzymes oxidize the methyl group to 5hmC, 5-formylcytosine and 5-carboxylcytosine (Figure 2). The 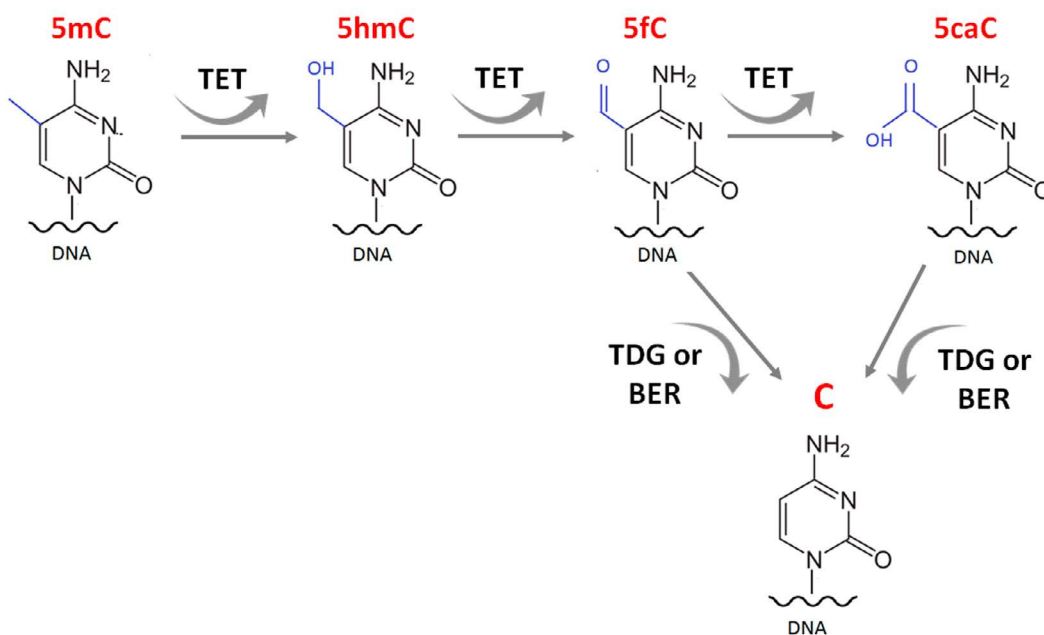bases are then recognized and excised by the enzyme thymine DNA glycosylase (TDG) and substituted by an unmodified cytosine by base excision repair (BER) [3-5]. Independent of their catalytic activity, TET2 and TET3 are also associated with chromatin remodeling and H3K4me3 mark (Tri methylation of lysine 4 in histone 3), a histone mark indicative of transcriptionally permissive chromatin states [6].

During the initial phases of embryonic development, TET enzymes are differentially expressed in several tissues, and have a specific expression profiles for each cell type, since the beginning of gastrulation [7-11]. Expression levels of TET enzymes (TET1 and TET2) in embryonic stem cells of animal models showed that these enzymes play important roles in tissue control and maintenance at embryonic level [12,13]. During central nervous system (CNS) development, these enzymes take part in several important biological processes, influencing oligodendrocyte maturation and differentiation, and myelination of cells [14].

TET1 is highly expressed in early blastocyst cells, and it is crucial in regulating the proliferation of neural progenitor cells [15]. Therefore,
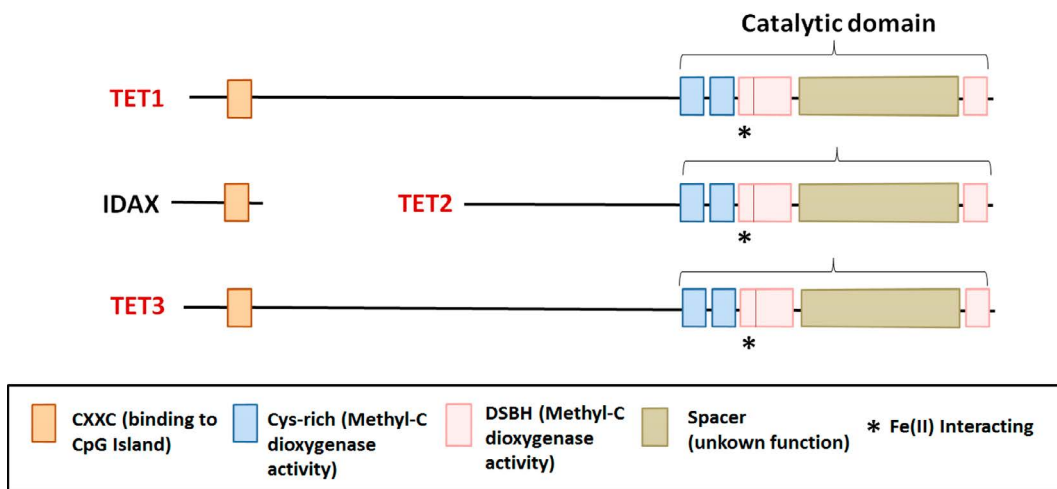
Figure 1. TETs showed a catalytic region in their C-terminal, with methylcytosine dioxygenase activity, composed by a conserved domain rich in cysteine (Cys-rich); and a double-stranded β-helix domain (DSHB), which interacts with 2OG and Fe (II). At the N-terminal region, TET1 and TET3 show a CXXC domain, which binds to unmodified cytosine; Conversely, as a consequence of a chromosome inversion during evolution, this domain is not present in TET2; the CXXC was separated and gave origin to the IDAX gene, which acts as its negative regulator

Figure 2. TET enzymes in the process of DNA demethylation. TET1/2/3 oxidize the methyl group into 5hmC, 5-formylcytosine and 5-carboxylcytosine. These bases are recognized and excised by the enzyme thymine DNA glycosylase (TDG) and substituted by an unmodified cytosine by base excision repair pathway (BER)

changes in its function can promote phenotype anomalies at the hippocampus and decreasing neurogenesis by transcription repression [15]. TET2 has been identified as responsible for maintaining hematopoietic stem cells and establishing DNA hypomethylation during neuronal tissues development. Likewise, TET3 is essential for the maintenance of neuronal progenitor cells in neocortical development [12,16-18].

Furthermore, TET1 and TET2 perform important functions during postnatal development. TET1 forms repressive complexes binding to target gene promoters, assuming an important role in transcriptional activation of tissues in development; [13] while the absence of TET2 may impair the maintenance of hematopoietic stem cells contributing to the development of acute myelomonocytic leukemia [12,19]. Therefore, TET enzymes activities are important in several stages of cell differentiation in tissue development, and any dysregulation, can lead to complications and loss of specific functions in each cellular context [20-22].

In cancer, decreased expression of *TET* genes and the consequent reduction of 5hmC levels have been frequently reported in tumors of different origins, and related to a poor prognosis [23-31]. Low 5hmC and 5mC levels are associated with the accumulation of methylation in specific promoter regions of tumor suppressor genes related to cancer progression [25,32,33]. Moreover, decreased 5hmC levels are associated with increased genomic instability [34].

A recent study demonstrated a similarity between the DNA methylation patterns of TET-deficient cells types and the profile of cancer cells, suggesting that local hypermethylation in euchromatin and DNA hypomethylation in heterochromatin can be associated with oncogenic transformation [35].

Manipulation of TET expression during embryonic development and in cancer cell lines has demonstrated the relevance of these genes in the control of conserved signaling pathways, such as Wingless (WNT), Notch, Sonic Hedgehog (SHH) and Transforming Growth Factor Beta (TGF-β), whose dysregulation is generally associated with tumor progression. In this review, we propose to analyze the action of TET enzymes in these signaling pathways in development, as well as the alterations displayed in cancer cells.

TETS and signaling pathways in embryonic development and cancer

TETs and WNT pathway

The WNT canonical signaling causes the accumulation of β-catenin in the cytoplasm and its eventual translocation into the nucleus to act as a transcriptional coactivator of transcription factors. When inactive, β-Catenin stability is regulated by a destruction complex (DC) [36,37]. Consisting of AXIN, the tumor suppressor Adenomatous Polyposis Coli (APC) and two activated kinases, the GSK3β (Glycogen Synthase Kinase 3 Beta) and CK1α/γ (Casein Kinase 1 Alpha/Gama). This complex, leads to the phosphorylation of β-Catenin, by CK1α/γ and GSK3β, and consequent proteasomal degradation. WNT ligands binding to receptor co-operators of Frizzled (FZD) and the lipoprotein receptor-related protein5/6 (LRP5/6) [38] and leads to their dimerization and the phosphorylation of the intracellular tail of LRP5/6 by GSK3β at serine 9. Consequently, the DC is abrogated and β-Catenin accumulates in the cytoplasm and nucleus where it binds to TCF/LEF (T-cell factor/lymphoid enhancer factor) and activates the transcription of WNT target genes: *AXIN2* and leucine rich repeat

containing G protein-coupled receptor 5 (*LGR5*), the key effectors of WNT signaling [39,40].

In tissue development, this pathway is constitutively activated until it reaches the precise stage and differentiation [41]. However, inactivating events on the DC or mutations in phosphorylation coding sites of genes belonging to WNT core components, lead to several pathologies, including cancer [41]. In recent years, WNT pathway components have frequently been described as over- or underexpressed in different human cancers playing key roles in tumor initiation, tumor growth, cell senescence, cell death, and metastasis [42]. In medulloblastomas, for example, the WNT canonical pathway is often activated and patients are categorized within the WNT molecular subgroup; and are assigned with a good prognosis [43]. Likewise, in breast and colon carcinomas, high levels of nuclear β-Catenin are usually considered predictors of increased WNT signaling activity and correlate with poor prognosis [44,45].

Alternatively, loss of APC is a driver event in colorectal cancer (CRC) and is associated with poor prognosis. Notably, tumor phenotype is reversed to normal as demonstrated by APC knockout *in vivo* models of CRC [46,47]. In other tumors, i.e. melanoma, the WNT pathway seems delays senescence [48], and mutations that disrupt the phosphorylation and degradation of β-Catenin are frequent in hepatocellular carcinoma and ovarian cancer, whereas mutations in *AXIN1* are common in colorectal tumors, most of which serve as prognostic indicators [49,50].

The investigation of *Tet1* deletions in mice also showed that this gene is required to maintain the morphology of epithelial cells and regulate the WNT pathway. *Tet1* absence resulted in increased 5mC levels and decreased 5hmC levels at the promoter regions of *Axin2*, *C-Myc* and *Sox-9* genes in intestinal stem cells isolated from mice [51]. Tet3 also displayed key roles in the regulation of the WNT pathway, controlling the balance between mesodermal and neuroectodermal fate in mouse embryonic stem cells on the generation of neural structures [52]. In mouse embryos, Tet3 demethylates the promoter region and activates the expression of *secreted frizzled-related protein 4 (Sfrp4) gene*, which is responsible for inhibiting the WNT pathway [52]. Moreover, the combination of transcriptome techniques and pharmacological manipulation, proved that Tet2 and Tet3 regulate the Notch and WNT pathways in zebrafish [53], and their inactivation during retinal neurogenesis results in the absence of a clear "zone" of differentiation and upregulation of *Wnt9b* [53].

Alternatively, in human cancer, the action of TET1 enzyme was demonstrated upstream the WNT pathway, and regulate *DKK* (*Dickkopf*) and *SFRP* genes expression by demethylation, both repressor genes of this pathway. In colon cancer, *TET1* gene knockout increases proliferation, while its induction, inhibits the proliferation and growth of xenograft tumors [54]. TET1 maintains the inhibitors of the pathway hypomethylated; its upregulation induces the activation of *DKK3* and *DKK4*, and the downregulation of *Myc* and *CyclinD2* [54]. Moreover, chromatin immunoprecipitation assays demonstrated that the TET1 binding at the promoters of *DKK* genes, as well as increased levels of 5hmC and the concomitant decrease in 5mC [54]. In epithelial ovarian cancer, the expression of *TET1* was inversely correlated with the clinical advance of the disease; while its upregulation inhibited colony forming, cell migration and invasion in SKOV3 and OVCAR3 cell lines, and EMT in SKOV3. Additionally, TET1 inhibited the WNT/β-Catenin pathway (or canonical WNT pathway), through the demethylation and upregulation of sFRP2 and DKK1 (Figure 3A) [55].

Tumor progression in melanoma was also associated with changes in 5hmC levels, and the enrichment of pathways by the Kyoto Encyclopedia of Genes and Genome evidenced the accumulation of 5mC in genes associated with the WNT pathway [25]. In medulloblastoma, the subgroup that displays activation of the WNT pathway showed lower expression of *TET1*, pointing out a possible association between its downregulation and the activation of the pathway [56].

TETs and SHH pathway

The canonical Hedgehog (HH) pathway consists, mainly, of the glycoproteins Sonic Hedgehog (SHH), Indian Hedgehog (IHH) and Desert Hedgehog (DHH) [57]. When SHH is secreted, it binds to the 12-transmembrane protein Patched-1 (PTCH1), a Smothened (SMO) inhibitor. SHH binding promotes abrogation of Patched-1 activity, releasing SMO. This event leads to the nuclear localization of glioma-associated transcription factors (GLI1, GLI2 and GLI3), considered the key effectors of this pathway [58].

Another important component of the SHH pathway, and repressor of GLI, is the suppressor of Fused (SUFU). SUFU is a negative regulator of the SHH pathway that directly binds to GLI, causing its arrestment, processing or degradation in the cytoplasm [59]. This mechanism prevents activation of SHH pathway target genes [60]. GLI activity can be involved in the activation of several genes including *CCND1* (*Cyclin*-D1, cell cycle), *MYC* (Proliferation), *BCL2* (Apoptosis), *ANG1*/2 (Angiogenesis), *SNAIL* (EMT), *NANOG* and *SOX2* (Stem cells self-renewal); and in the positive feedback loop stimulating transcription of *GLIs* and *PTCH1* [61-65].

In cancer, the SHH pathway is aberrantly activated due to mutations in *PTCH1*, *SUFU* or *SMO*. Persistent activation of the pathway stabilizes the expression of *GLI1* and *GLI2* that promotes proliferation and invasion and inhibits apoptosis [58].

Specifically in medulloblastoma, the SHH pathway plays an important role in the molecular subgroup assigned as SHH-medulloblastoma (SHH-MB) [58]. In rhabdomyosarcoma, the most frequent soft tissue sarcoma in children, which is divided into two major histological subgroups, embryonal and alveolar, is described to show HH/SMO pathway activation [65]. Embryonal Tumors are characterized by SMO localization in primary cilia and/or GLIs hyperexpression. These features are almost exclusively found in tumors with activated SHH signaling pathway [43,65]. Clinical trials are being conducted to test SMO inhibitors, such as Vismodegib or Sonidegib. Nonetheless, despite initial favourable outcomes, some patients still present resistance or relapse [43].

SHH is also controlled by TET enzymes. Xu et al. [66] demonstrated the role of Tet3 5mC hydroxylase and its CXXC domain in upstream transcriptional regulation of genes important in early eye and neural development, through loss-of-function studies focused on Tet3 and using *Xenopus sp.* as an experimental model. Depletion of Tet3 exhibited effects in a set of key development genes, including, two major SHH signaling components: *shh* and *ptc-1*, that showed low expression in embryos at stage 14, in the absence of *Tet3* [66]. Moreover, chromatin immunoprecipitation (ChIP) assay demonstrated the binding of Tet3 enzyme in promoter region of *shh* and *ptc-1* [66] (Figure 3B). In parallel, regulation of the 5hmC status at its targets showed a significant reduction in 5hmC at CCGG sites in *ptc-2* gene promoter, after Tet3 depletion [66]. Of note, the CXXC domain of TET3 has DNA interaction and binding to restricted genomic regions, including the *PTCH1* and *SHH* genes in HEK293T cells [66].

The control of SHH by Tets can also occur indirectly. Tet1/Tet3 knockout, in mice (*Tet1/3* DKO embryos), induced defects in cholesterol synthesis; since SHH signaling requires covalent modification of cholesterol for its activity during development. Significant decrease in expression of SHH components, such as *Ptch1*, *hedgehog interacting protein (Hhip), transcription factors (Gli1/2, Pax1/9)* and other pathway targets, such as *Cyclin D2 (Ccnd2)* was also observed in this model (Figure 3B). Dysregulation in SHH function contributes to developmental abnormalities [67]. Late stage *Tet1/3* DKO exhibited poor forebrain formation and abnormal facial structures, with phenotypes observed in the dysfunction of SHH [66]. Thus, the function of TETs enzymes is fundamental for the SHH pathway activation during development. In cancer, medulloblastoma cell lines classified as SHH subgroup showed high levels of *TET1* expression [68]. However, there are no functional descriptions of TET and SHH or about any correlation between them in other tumor types.

TETs and TGF-β

The transforming growth factor-β (TGF-β) family consists of three TGF-β isoforms (TGFB1, TGFB2 and TGFB3), NODAL, bone morphogenetic proteins (BMPs), and growth and differentiation factors (GDFs) [69]. These proteins share features regarding signaling, regulation and structure, however, the specific function and complexity of the canonical and non-canonical pathways remain to be elucidated [69-72].
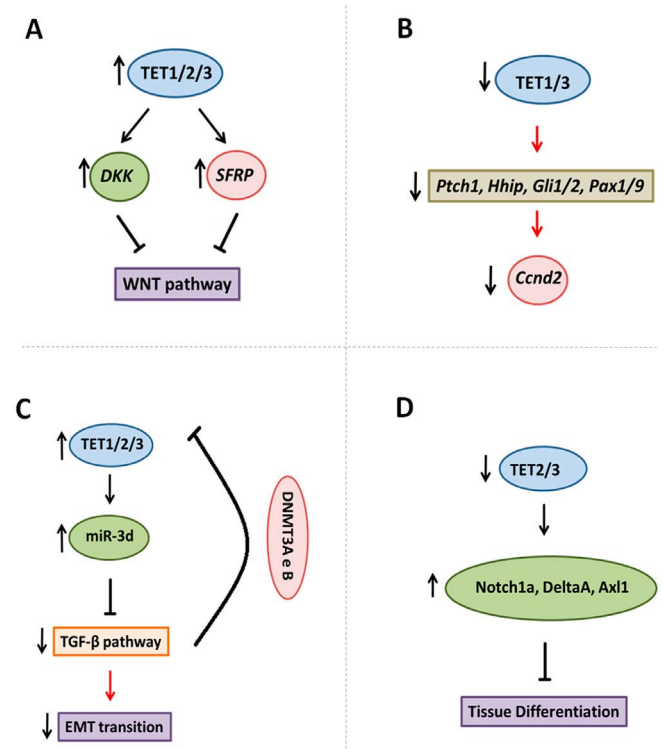
Figure 3. TET enzymes target signalling pathways. A. TET enzymes demethylated DKK and SFPR genes that inhibited WNT pathway in embryonic development and cancer. **B.** Tet1/3 knockout is associated with decreased *Ptch1, Hhip, Gli1/2 and Pax1/9* genes, that are members of SHH pathway, and reduced downstream genes such as Ccnd2. **C.** TET3 demethylates the precursor of miR-3d, and thus, inhibiting TGF-β pathway, and reducing epithelia-mesenchymal transition. Whereas TGF-β pathways stimulated DNMT3A and B activity in promoter of TET2 and 3, with a reduced in these genes expression. **D.** The control of NOTCH signaling components by Tet2 e Tet3 enzymes.

The activation of this pathway requires the ligand binding to one of the five membranous type II serine/threonine kinase receptors and recruitment and transphosphorylation of one of the seven type I serine/threonine kinase receptors [69]. After activation by phosphorylation, the type I receptor kinase domain triggers the signal of the canonical pathway, which comprises the phosphorylation of the SMAD-dependent axis, including receptor-regulated SMAD proteins (R-SMAD) [69]. During the canonical signaling, SMAD2 and SMAD3 with NODAL, activin and TGFB are consistently correlated. Alternatively, some authors also describe that TGFB possibly induces phosphorylation of SMAD1 and SMAD5, as alternative signals triggered by BMPs and GDFs [70,72]. During downstream signaling, SMAD2 and SMAD3 form complexes and translocate into the nucleus after phosphorylation. In the nucleus, after accumulation and binding to cofactors, such as p300-CBP and FOXH1, they trigger transcription of several target genes [73].

Other potential regulators of TGF-β pathway are the Mitogen Activated Protein Kinases (MAPKs), Glycogen synthase kinase 3β (GSK3β), cyclin dependent kinases (CDKs) and Smurf (SMAD ubiquitination regulatory factor). Altogether, they can modulate SMADs activities in different manners, including phosphorylation and ubiquitination [69].

The TGFB family components are involved in early embryonic development and tissue homeostasis in adults. Nonetheless, their roles in cellular growth, differentiation, apoptosis, extracellular matrix production, EMT and immune-response, have only been partially elucidated [69]. However, when aberrantly activated or inactivated, depending on the cellular context, they might promote different pathologies [74].

Regarding carcinogenesis and tumor initiation, the TGF-β pathway shows duality: in the early stages, it might act as a tumor suppressor, though later, it might stimulate a pro-carcinogenesis microenvironment, promoting in most cases, metastasis [74]. Several studies have demonstrated that *TGFBR1, TGFBR2, SMAD2* and *SMAD4* are frequently inactivated through mutation, allelic heterozygosity loss or inactivation through methylation [70]. In SHH medulloblastoma, high *SMAD2* expression and IHC staining in patients samples was associated with good prognosis while, in Group 3 medulloblastoma, the SNAIL/TGF-β axis is associated with metastasis and promotes EMT [75]. In others tumors (i.e., melanoma, renal cell carcinoma, mesothelioma, glioma and pancreatic ductal adenocarcinoma) there are several ongoing clinical trials utilizing TGF-β inhibitors to target TGFB1 or TGFB2 or both [74]. Fresolimumab, a TGF-β inhibitor for TGFB1, B2 and B3 and PF-03446962(®Pfizer) an ALK inhibitor, are the most widely used. Nonetheless, despite the initial good outcomes, the authors suggested the combination of TGF-β inhibitors with the current standard chemotherapy or epigenetic modulators to improve the efficiency of multimodal therapy [74].

In ovarian cancer, TGFB1 induced EMT with downregulation of miR-30 family and the restoration of miR-30d inhibited this phenotype. Moreover, the action of TET3 in TGF-β pathway in this model, occurred through the demethylation of miR-30d precursor [76]. Furthermore, when ovarian cancer cell lines SKOV3 and 3OA were treated with TGF-β1 (10ng/mL), they showed downregulation of TET1 and TET3, although, no alteration in TET2 was observed. TET3 was the most significantly reduced and when upregulated inhibited the TGF-β pathway and resulted in the blockade of EMT with the

concomitant reduction of the EMT markers, E-cadherin, Vimentin, N-cadherin and Snail. Therefore, cells with TET3 expression exhibited decreased invasive and migratory capabilities [76]. Also, it has been suggested that the TGF-β pathway could regulate *TET2* and *TET3* genes expression, by stimulating the expression and recruitment of DNMT (DNA methyltransferase) 3A and 3B to their promoter regions, inducing hypermethylation and consequent silencing of these genes (Figure 3C) [77].

TETs and notch pathway

The Notch signalling pathway is critical for embryonic development. This pathway is activated by interactions between adjacent cells in order to contribute to the formation, growth, and development of embryos [78]. Even though Notch was first classified as a neurogenic gene, the characterization of Drosophila embryos made it clear that this pathway is highly pleiotropic [79]. Notch signaling has been described to be activated in somite formation (somitogenesis), heart formation (cardiogenesis), muscle tissue formation (myogenesis), hematopoiesis, vasculogenesis and angiogenesis, among others [78,80,81].

In mammals, there are four Notch receptors, NOTCH 1-4, and five transmembrane ligands, Jagged1 (JAG1), Jagged2 (JAG2), Delta-like1 (DLL1), Delta-like3 (DLL3), and Delta-like4 (DLL4), which interact with each other to activate the pathway. At the molecular level, the binding between Notch receptors and their ligands promotes two proteolytic cleavage events at the receptors. The first cleavage is catalysed by the ADAM-family of metalloproteases and the second cleavage is mediated by ɣ-secretase, an enzyme complex that contains PRESENILIN, NICASTRIN, PEN2 and APH1. These cleavages release the Notch intracellular domain (NICD), which translocates into the nucleus and acts as a transcriptional coactivator. NICD cannot bind directly to DNA but heterodimerizes with the DNA-binding protein RBP-J (recombination signal binding protein for immunoglobulin kappa J)/CBF1, forming the CSL complex (CBF1, Su(H) and LAG-1), thus, activating the transcription of Notch-target genes [82].

Notch pathway was described for the first time in leukemia, however, is also involved in other tumors. In breast cancer, low expression of NUMB, a negative regulator of Notch pathway, and an increased NOTCH1 expression were described in approximately 50% of primary human mammary carcinomas [83]. Likewise, in medulloblastoma, higher expression of *NOTCH2* was shown in 15% of the cases and high expression of *HES1*, a target of Notch signaling, was associated with decreased survival [84].

TETs enzymes and 5hmC, also play relevant roles in controlling the expression of molecules involved in the Notch signaling pathway. Some studies have demonstrated a relationship between Notch signaling genes and epigenetic modifications in biological processes. Notch pathway plays a critical role in osteoblast cell fate and function, and endothelial Notch activity is associated with stimulation of angiogenesis and osteogenesis [85]. It was reported that TET3 and 5hmC were upregulated in dexamethasone-treated MLO-Y4 osteocyte-like cells and pathways enrichment analysis by KEGG showed that Notch signaling pathway exhibited dynamic changes in 5hmC levels and showed the most significant 5hmC downregulation, among the other signaling pathways, in those cells [86]. Changes in NOTCH4 expression were also found in steroid-associated osteonecrosis tissue suggesting that Notch signaling may also partially mediate the functional effect of 5hmC changes [86].

During differentiation of embryonic midbrain-type neural precursor cells derived from human embryonic stem cells occurred

an increase of *NOTCH1* expression, with gains in 5hmC at the gene body and a loses of 5mC at the promoter and gene body. Besides that, the NOTCH1 signaling pathway target genes *DLL1*, *HES5*, *DNER* and *GFAP* also gained 5hmC [87]. Moreover, significant hypomethylation was observed using bisulfite sequencing in myoblasts, myotubes, and skeletal muscle when compared to non-muscle samples at intragenic or intergenic regions of the following Notch receptor or ligand genes: *NOTCH1*, *NOTCH2*, *JAG2*, and *DLL1* [88]. In parallel, an enzymatic assay of sites within or near these genes revealed an unusually high enrichment of 5hmC (up to 81%) in skeletal muscle, heart, and cerebellum [88]. Moreover, a study from Li, C et al. [89] demonstrated that Notch signaling in the hemogenic endothelium is regulated by Tet2/3 [89], while, it was [53] showed that occur the control of Notch signaling by Tet2 e Tet3 during zebrafish neurogenesis. In the absence of *Tets*, there is an upregulation of *Notch1a, DeltaA, Axl1* genes (Figure 3D); and in Tet2 and Tet3 mutants the retina did not differentiate properly [53].

Conclusions

In this review, we discussed the role of TETs enzymes and their association with signaling pathways in embryonic development and cancer. The expression of TETs is fundamental for normal embryonic development and their deletion in animal models has shown to delay cell differentiation and result in dysregulated expression of genes involved in signaling pathways. Consequently, the absence of TETs results in central nervous system defects and retinal deformity. In cancer, low expression of TETs induces activation of the WNT, TGF-β and NOTCH pathways, either directly or indirectly. Depletion in Tet activity inhibits tumorigenic processes, such as cell proliferation and epithelial-mesenchymal transition (EMT). The prospect of TET pharmacological or molecular manipulation might have global effects that should be considered for future therapeutic intervention.

Declarations

Availability of data and material: Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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