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REVIEW

TENT5/FAM46: An Enigmatic Family of Secretory Tuners

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ABSTRACT

Human TENT5 family comprises four members (A–D) associated with different diseases of secretory cells. Homozygous mutations in TENT5A cause a rare form of osteogenesis imperfecta due to impaired collagen deposition by osteoblasts. TENT5C is frequently mutated or deleted in patients with multiple myeloma, the cancer of antibody-secreting plasma cells, and TENT5D alterations result in male infertility. TENT5 members are noncanonical poly(A)polymerases that selectively stabilize mRNAs encoding endoplasmic reticulum–imported proteins, thus promoting the expression of secretory cargoes and proteins involved in folding, glycosylation, and trafficking along the secretory apparatus. This specificity has been proposed to be linked to TENT5 localization at the membrane of the endoplasmic reticulum, thanks to their interaction with transmembrane FNDC3 proteins. Recently, key roles of TENT5 proteins have been described in cancer, bone homeostasis, immunity, stemness, and fertility. This review will comprehensively analyze the identified cellular functions of this novel family of secretory tuners in physiological and pathological conditions, highlighting the proposed molecular mechanisms and the remaining open questions.

1 | Evolution and Characteristics of TENT5/FAM46 Family

Terminal nucleotidyl-transferases group (TENTs) encloses several polymerases catalyzing the 3′-tail extension of RNA molecules, a key process modulating RNA stability and eventually protein synthesis [1]. The human genome encodes for eleven TENTs subdivided by phylogenetic analysis into six subfamilies (TENT1–6) [1]. TENTs include terminal uridylyl transferases (TUTases) and noncanonical poly(A)polymerases (ncPAPs) (reviewed in [1]). NcPAPs are less characterized polyadenylation enzymes than the widely described canonical PAPs that provide the 3′ poly(A) tailing of mRNAs in the nucleus [2, 3]. NcPAPs and TUTases share with canonical PAPs a conserved catalytic domain and a similar mechanism of action; however, in TENTs, with the exception of TENT1, the RNA-binding domain (RBD) is missing [1].

Terminal nucleotidyl-transferase 5 (TENT5) family, also known as family with sequence similarity 46 (FAM46), is a highly conserved group of cytoplasmic ncPAPs [4].

Actual TENT5s probably appeared in the Unikonta ancestor; indeed, Metazoa, Choanoflagellida, and Amoebozoa have at least one copy, which has been lost in Fungi and Plants. Apart from fishes having six or seven copies, vertebrata carry four copies of TENT5 genes [5]. Human TENT5 family consists of four members (TENT5A–D) expressed in various tissues, except for the testis-specific TENT5D. The phylogenetically closest members are TENT5A and TENT5C, while TENT5D is the most evolutionarily distant member [6] with the lowest sequence similarity (Table 1).

Human TENT5s are part of the heterogeneous nucleotidyl-transferase (NTase) fold superfamily, whose members, despite

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high sequence variability, share a common active domain with a α/β-fold structure composed of a three-stranded β-sheet and four α-helices (topology: αβαβαβα) [3, 7]. Accordingly, all TENT5s possess an N-terminal α/β NTase domain responsible for the PAP activity followed by a helical domain (HD) which resembles a PAP/OAS1 substrate-binding domain [5]. In addition to these two conserved domains, two terminal tails with a more disordered structure, generate most of the variability between TENT5 members. The N-terminal tail determines the difference in length since it varies from 50 to 70 amino acids (aa) in TENT5A and TENT5B to be almost absent in TENT5D. On the contrary the C-terminal tail is responsible for the greatest variability of TENT5D when compared with the other members. The NTase domain is characterized by a key motif, three aspartate/glutamates located on the second and third β-strands, fundamental for the coordination of divalent ions and for the polyadenylation activity. Moreover, a hG[GS] pattern, where "h" indicates a hydrophobic residue, placed before the catalytic aspartates/glutamates, is fundamental to hold the substrate within the active site [3, 7]. While the active sites of the NTase domain have been defined and characterized, the molecular features of the HD are more elusive.

The PAP activity makes these proteins key regulators of mRNAs stability, thus promoting the translation of the encoded proteins and significantly influencing the cellular proteome. Notably, TENT5-mediated polyadenylation is not indiscriminate since TENT5s have a selectivity for mRNA of proteins targeted to the secretory compartment [8–11]. This substrate specificity has been proposed to be mediated by TENT5s localization at the endoplasmic reticulum (ER) membrane, thanks to their interaction with ER-transmembrane FNDC3 proteins, and to the presence of a sequence for the signal peptide in the target mRNAs (Figure 1) [9, 12]. In line with this selective role in the secretory trafficking, the conserved TENT5 homolog in *Caenorhabditis elegans* (PQN-44) was found to increase the stability of mRNAs encoding for anti-microbial immune response factors to increase their release in the surrounding environment [6]. Consequently, TENT5-depleted worms showed a shorter survival than the wild type when exposed to bacterial infection [6]. Similarly, as extensively described in the following dedicated sections, human TENT5s have been described to be rapid and potent tuners of protein secretion in different tissues and have been associated with different alterations of secretory cells. Indeed, TENT5A mutations cause a rare form of osteogenesis imperfecta (OI) due to insufficient collagen I deposition [11, 13], TENT5C modulates immunoglobulin secretion in plasma cells [8–10, 14], and is frequently mutated in their malignant counterpart, multiple myeloma [15], and loss of TENT5D results in male infertility [16–18].

TABLE 1 | Identity percentage among TENT5 family members.

Comparison	Identity percentage
TENT5A vs. TENT5C	72.89%
TENT5A vs. TENT5B	65.28%
TENT5B vs. TENT5C	63.61%
TENT5A vs. TENT5D	62.91%
TENT5C vs. TENT5D	59.63%
TENT5B vs. TENT5D	52.46%

In this review, we will comprehensively describe the scientific literature on TENT5 proteins, to summarize the current knowledge on their expression, mechanisms of action, and their physiologic and pathologic roles in development, stemness, immunity, cancer, and fertility.

2 | TENT5A/FAM46A

TENT5A gene is located on chromosome 6 (6q14.1) and is formed by three exons. It generates a transcript that undergoes alternative splicing, leading to the production of two isoforms

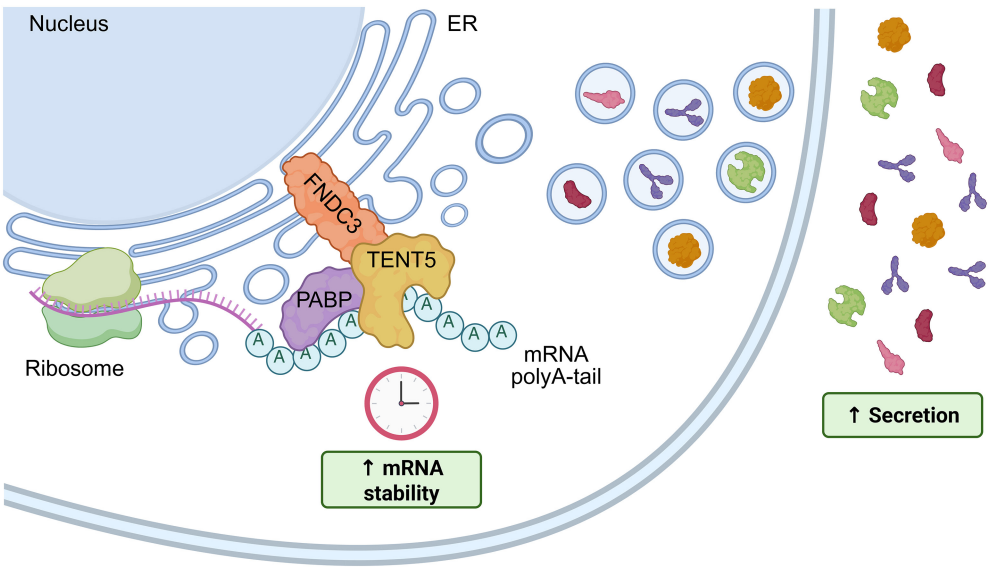

FIGURE 1 | TENT5s are noncanonical poly(A)polymerases localized at the ER-membrane where they stabilize mRNAs encoding for proteins targeted to the secretory apparatus.

of 442 and 461 residues respectively (UniProt: Q96IP4-1 and Q96IP4-2), which differ for their N-terminus. TENT5A gene is widely expressed in many tissues, with a preference observed in salivary glands and bone marrow (Human Protein Atlas), and encodes a nuclear or cytoplasmic ncPAP, with its localization depending on tyrosine phosphorylation status [19]. In the nucleus, TENT5A mainly localizes in euchromatin regions, whereas in the cytosol, it interacts with FNDC3 proteins at the ER membrane [9, 19].

TENT5A has been reported to be involved in bone development and postnatal bone homeostasis [11, 13, 20]. Loss-of-function mutations are reported in patients with a rare form of autosomal recessive OI (OI type XVIII, OMIM # 617952) [13]. In three different families, TENT5A mutations causing severe OI were identified by Doyard et al.: two homozygous deleterious variants (H127R and D231G) and a third frameshift mutation resulting in a premature stop codon (S205Yfs*13). OI patients are usually affected by growth delay, bone fragility, and frequent fractures [21]. In most cases, OI is caused by defects in collagen I genes, COL1A1 and COL1A2; however, other forms are due to alterations in genes involved in collagen folding, processing, and trafficking along the secretory route, or in factors playing a role in osteoblast differentiation and bone mineralization [22]. Accordingly, TENT5A is highly induced during osteoblast differentiation, when it mediates a wave of cytoplasmic polyadenylation essential for abundant collagen secretion [11]. Of note, Gewartowska et al. generated a TENT5A knockout (KO) mouse model by introducing a frameshift mutation that alters the NTase domain of murine TENT5A. In the osteoblasts of these mice, COL1A1 and COL1A2 mRNA levels were decreased due to a significant shortening of their 3′ tails [11]. Interestingly, alteration in collagen synthesis is peculiar to type I OI, the mildest form of the disease, whereas the TENT5A-caused type XVIII OI is associated with a severe phenotype, suggesting that low levels of collagen I may not be the sole factor contributing to the disease in these patients [13]. In line, Gewartowska et al. demonstrated that the KO also interferes with normal osteoblast development and bone mineralization, reducing the polyadenylation of essential mRNAs, like Sparc and SerpinF1, two other OI-causative genes [11]. Furthermore, mutations in the NTase domain were also found in a spontaneous mouse model (BAP014) characterized by skeletal dysplasia, carrying a nonsense mutation, leading to a premature stop codon in the position 156 of TENT5A (E157*) [20]. BAP014 heterozygous mice display higher serum alkaline phosphatase (ALP), an index of bone resorption; while the few homozygous mice escaping embryonic lethality have reduced body size, abnormal gait, altered bone mineralization, and bone fragility [20]. Overall, the clinical observations in OI patients and in two independent mouse models confirm a crucial role of TENT5A in bone development and homeostasis.

Notably, the TENT5A gene is characterized by the presence of a variable number of tandem repeats polymorphism (VNTR) in exon 2, consisting of two to seven repeats of 15 bp [13, 23]. Interestingly, this VNTR polymorphism is associated with human height [23] and with increased susceptibility to multiple disorders: hip and knee joint osteoarthritis, tuberculosis, NSCLC, retinitis pigmentosa, and colorectal cancer [24–28]. TENT5A may also be involved in the pathogenesis of adolescent idiopathic scoliosis (AIS) by regulating the formation of Type I muscle fibers. Indeed, TENT5A shows a differential expression in the two-sided paravertebral muscles from AIS patients, and its knockdown was shown to inhibit the myogenic differentiation of murine myoblast C2C12 cells [29]. In line, tissue expression of TENT5A is significantly lower in AIS patients compared with controls, and it is correlated with patient bone mineral density [30].

Beyond its relevance in bone homeostasis, TENT5A has an evolutionarily conserved role in innate immunity, as described in *C. elegans* [6], being expressed along with TENT5C in macrophages. In these cells, TENT5s stabilize mRNA encoding proteins of the extracellular space, lytic vacuoles, or involved in defense responses [6]. Notably, the polyadenylating activity of TENT5 proteins in macrophages has also been proposed to enhance the stability and immunogenicity of SARS-CoV-2 mRNA vaccines by counteracting the gradual deadenylation of the vaccine [31]. These findings significantly increase the translational relevance of this family of proteins, as mRNA-based vaccines represent a crucial strategy for future immunization programs.

On the contrary, the role of TENT5A in cancer is still controversial with no identified causative mutations. However, its expression has been associated with glioma, ovarian cancer, esophageal adenocarcinoma, and breast cancer [32–38]. TENT5A was found highly expressed with a pro-tumoral role in glioma [32] and has been functionally linked to abnormal activation of the Wnt/β-catenin pathway, influencing glioma prognosis and progression [34]. In addition, its expression in ovarian cancer was associated with an aggressive phenotype and chemotherapy resistance [38]. In line, TENT5A silencing was found to synergize with cisplatin treatment in vivo through the modulation of the TGF-β/SMAD pathway [35]. TENT5A-mediated regulation of the TGF-β/SMAD pathway may also provide a deeper insight into TENT5A's role in bone since alterations of this pathway are associated with skeletal dysplasia in humans. In particular, TENT5A has been shown to physically interact with SMAD1 and SMAD4 to stabilize them, promoting in this way the expression of BMP and bone growth [39, 40]. Finally, it has been proposed a possible contribution of TENT5A in pregnancy and postpartum depression [41, 42]. Altogether, these clinical and experimental data revealed a defined role of TENT5A in skeletal development and bone homeostasis while its involvement in other biological processes and diseases is still unclear.

3 | TENT5B/FAM46B

TENT5B gene is located on chromosome 1 (1p36.11) and contains two exons encoding for a 425aa-long protein. TENT5B is expressed in many adult tissues with increased levels in esophagus and skin (Human Protein Atlas). Hu et al. solved a 2.7A crystal structure of TENT5B from *Xenopus tropicalis* showing a similar topology to the class II bacterial PAPs. The NTase catalytic site is composed of D91, D93, and E167 (D125, D127, E201 in human) preceded by the conserved hG[GS] motif [43]. TENT5B is selective for 3′-end adenosine-rich RNA with a preference for substrates ending with an AxAA sequence [43]. TENT5B has been described to possess higher

PAP activity than TENT5C [43]; however, having the lowest affinity for FNDC3 proteins, TENT5B has reduced localization at the ER membrane when compared with the other TENT5s [9]. Accordingly, its expression in multiple myeloma cells results in negligible effects on the secretory apparatus and on antibody production [9].

Interestingly, TENT5B promoter contains OCT4 and NANOG binding-sites, suggesting a possible role in stemness and embryonic development. In line, its expression is elevated in human embryos and stem cells (hESCs and iPSCs) and decreases during neuroectoderm and mesendoderm differentiation and embryoid body formation, while the mRNAs of the other members remain unaltered [43–45]. In line, TENT5B constitutive KO is lethal while its knock-down in hESCs induces apoptosis [43]. In hESCs, TENT5B conditional KO decreases the mRNA levels of WNT5A, NANOG, RICTOR, and SKP2, suggesting a specific role of TENT5B in preventing the degradation of stemness-related mRNAs [43].

However, the role of TENT5B in organismal development and stemness remains controversial. TENT5B KO mice are viable without noticeable phenotypic abnormalities [12]. Similarly, double KOs of TENT5B and TENT5C are also viable but show defects in gametogenesis, which can be rescued by the expression of either protein [12]. Interestingly, TENT5B-GFP knock-in mice also exhibit fertility defects but only when the GFP is fused at the C-terminus. This fusion leads to a gain-of-function effect, increasing TENT5B stability, likely by inhibiting its ubiquitination [12]. In line with a role in gametogenesis, Anbazhagan et al. showed how TENT5B expression is modulated by GRTH, an essential factor in spermatogenesis [46].

A possible antitumoral function of TENT5B in several cancers has been investigated. Its deregulation was found in prostate cancer, non–small cell lung cancer (NSCLC) and metastatic melanoma [47–50]. In patients with prostate cancer, TENT5B overexpression inhibits malignant cell proliferation in vitro and in vivo [47]. Paclitaxel-resistant prostate cancer cells showed downregulated TENT5B expression, suggesting a possible role in the sensitivity to chemotherapy [49]. Sang et al. showed similar results in NSCLC cells [50]. In both cancers, TENT5B downregulation was associated with the activation of the β-catenin pathway. Indeed, in prostate cancer cells, β-catenin was upregulated due to the inhibition of its ubiquitination [47]; whereas, in NSCLC cells, the treatment with a Wnt/β-catenin inhibitor ameliorated the TENT5B knockdown phenotype [50]. TENT5B was also shown to influence glycolysis and apoptosis of prostate tumor cells through the regulation of glucose uptake and LDHA activity via MYC activation [48]. In NSCLC cells, TENT5B was found to regulate MMP7 and VEGF [50]; whereas its expression is modulated by MLL4 [51].

Beyond cancer, TENT5B has been proposed as a prognostic marker in refractory lupus nephritis since its expression was lower in the kidneys of non-responders to immunosuppressive drugs [52]. Overall, TENT5B remains the most elusive member of the family with a cellular role not completely defined and without a clear causative association with any human disease.

4 | TENT5C/FAM46C

TENT5C gene is on chromosome 1 (1p12) and is constituted by two exons. The gene encodes a 391aa protein that can be found in several tissues but especially in bone marrow, pancreas, and testis (Human Protein Atlas). TENT5C structure has been solved [53, 54]. Like the other members, the protein consists of two principal domains: a N-terminal NTase domain (10-229aa) followed by a HD (230-343aa), that form a central cleft. The NTase catalytic motif is formed by the conserved residues G73, S74, D90, D92, and E166. TENT5C is a weaker PAP than TENT5B despite the high sequence similarity [43]. This difference may be accounted for by conformational divergence and single residue changes in the NTase domain. Indeed, the replacement of TENT5C residues with TENT5B counterparts (G77S, T290R, D298G, and N72H) increased its PAP capacity [53]. TENT5C acts only on A-rich RNA oligos with a polyadenylation efficiency directly dependent on the length of the poly(A) tail at the 3′ end [53].

TENT5C gene is frequently mutated or deleted in up to 20% of patients with multiple myeloma [15, 55–58]. Loss-of-function mutations are distributed along the entire coding sequence, apart from the N-terminal portion, implying a tumor suppression activity [15]. Moreover, TENT5C locus is involved in translocations with chromosome 8 resulting in MYC overexpression boosted by TENT5C enhancers [59, 60]. Interestingly, smoldering multiple myeloma, an early asymptomatic manifestation of the plasma cell malignancy, is characterized by significantly fewer TENT5C mutations and deletions compared to multiple myeloma, suggesting a causal role in the transition from smoldering toward active multiple myeloma [61, 62]. TENT5C's oncosuppressive role in multiple myeloma has been linked with its PAP activity stabilizing mRNAs encoding for immunoglobulins and other ER-targeted mRNAs [8–10]. In this way, TENT5 proteins increase the expression of proteins responsible for ER import, folding, and glycosylation, thus potently promoting antibody secretion. Accordingly, TENT5C is induced by the plasma cell identity factor PRDM1/Blimp1 and by TLR activation during B to plasma cell differentiation to sustain humoral immunity [9, 14]. In line, Bilska et al. showed that TENT5C KO B cells fail to sustain proper antibody responses, despite higher differentiation rates to CD138-positive cells [14]. These data suggest that TENT5C physiologically promotes immunoglobulin production and secretion in plasma cells, whereas multiple myeloma cells aim at curbing its expression to “egotistically” decrease antibody synthesis and the related metabolic expenditure and degradative stress [63]. Indeed, TENT5 re-expression in mutated multiple myeloma cells induces a secretory boost coupled with a block in proliferation, ROS accumulation, reduced IRF4 and MYC levels, and increased apoptotic rates [9, 64]. On the contrary, its downmodulation results in an increase in cell proliferation and clonogenicity associated with the activation of the PI3K/AKT survival pathway [8, 9, 64, 65]. These results unveil a role of TENT5C as a key modulator in multiple myeloma pathobiology, balancing the secretory activity and tumor fitness and proliferation [66]. Notably, by modifying the secretory capacity, TENT5C has been also shown by us to modulate the surface expression of key receptors involved in the multiple myeloma-immune system crosstalk (calreticulin) or target of immunotherapy (CD38). This observation suggests a possible effect of TENT5C mutations in

modulating multiple myeloma immunogenicity and response to therapies [66].

However, this oncosuppressive model is still debated since some authors described PAP-independent antitumoral properties of TENT5C in multiple myeloma and highlighted its ability to modulate autophagy, the unfolded protein response (UPR) and protein aggregation [67]. The pleiotropic effect of TENT5C can also be justified by the different interactors modulating its localization, functions, and activity. The ER-transmembrane proteins FNDC3A and FNDC3B have been shown to mediate its localization on the ER-membrane, which is fundamental for TENT5C modulation of autophagy and protein secretion [9, 67]. Other TENT5C partners are linked to the PAP activity. TENT5 proteins lack the RBD but interact with two poly(A)-binding proteins (PABPC1 and PABPC4) [8, 9]. Moreover, Liu et al. have recently shown how BCCIPa can bind TENT5C to inhibit its PAP activity, hypothesizing a role of this interaction in regulating genome stability, cell cycle, and tumor development [68]. TENT5C also interacts with polo-like kinase 4 (PLK4), the master regulator of centrosome duplication. This binding inhibits PLK4 autophosphorylation and activation, thus reducing cell proliferation and cancer growth in a PAP-independent manner [54, 69]. Finally, TENT5C has been described to interact with p62, which in the case of altered proteostasis can sequester it in aggregates, preventing its association with FNDC3 proteins and its effects on the secretory apparatus [9].

Scientific interest in TENT5C arose from the identification of its tumor suppressive role in multiple myeloma (multiple myeloma); however, this ncPAP has been lately linked to other forms of cancer [70] and different biological processes such as spermatogenesis [71], diabetes [72], erythropoiesis [73], and macrophage polarization [74, 75]. In addition to multiple myeloma, TENT5C has been proposed to act as an oncosuppressor in other tumors such ashepatocellular carcinoma [76, 77], prostate cancer [78], gastric cancer [79–81], osteosarcoma [82], squamous cell carcinoma of the lung [83], chromophobe renal cell carcinoma [84], esophageal squamous cell carcinoma [85], and oral cell carcinoma [86]. In hepatocellular carcinoma, norcantharidin (NCTD) treatment was shown to induce TENT5C expression, inhibit cell proliferation, and increase apoptotic susceptibility [76]. Both NCTD and TENT5C overexpression result in reduced RAS levels, MEK1/2, ERK1/2 phosphorylation, and TGF-β/SMAD pathway activation [76, 77, 87]. Interestingly, these NCTD anticancer effects are weakened by TENT5C silencing, proving that the pharmacological activity of NCTD relies on TENT5C. In prostate cancer, TENT5C's inhibitory effect on tumor growth depends on its action on the PTEN/AKT pathway [78]. In gastric cancer, TENT5C antagonizes cell proliferation by interfering with the activation of Wnt/β-catenin and PLK4 [80, 81].

Beyond cancer, Zheng et al. found that TENT5C localized in the manchette of mouse spermatids, a region involved in cellular protein transport and reshaping [71]. A key role of TENT5C in gametogenesis has been recently confirmed by Brouze et al., who proved that TENT5C KO results in male sterility, probably due to altered modulation of TPPP2 and INSL3, and in female infertility when combined with the TENT5B KO [12]. An additional important biological function of TENT5C was identified in promoting erythropoiesis [73, 88]. Indeed, TENT5C KO mice and catalytic-inactive knock-in mice exhibit microcytic hypochromic anemia [8, 88]. TENT5C is highly expressed during the later stages of the erythroid lineage, where, in cooperation with the poly(A)-binding proteins LARP4/5, it counteracts the gradual deadenylation of globin mRNAs [73, 88].

TENT5C expression has been shown to be upregulated by INFα, INFγ, IL4, TLR receptor, and PRDM1 [9, 14, 89, 90]. On the contrary, various noncoding RNAs (ncRNAs) have been described to negatively modulate its expression. For instance, by inhibiting TENT5C, miR10-b increases osteosarcoma proliferation and invasiveness [82]; miR-1269a promotes esophageal squamous cell carcinoma [85]; while miR-296-5p, miR-324-3p, and miR-3928-3p induce MYC expression and tumor proliferation in lung squamous cell carcinoma [83]. Interestingly, TENT5C modulation through ncRNAs is also crucial in regulating macrophage polarization. Targeting TENT5C, miR-657 is responsible for promoting M1 polarization in gestational diabetes mellitus [74]; whereas Yang et al. described the role of circRNA_17725 in promoting M2 polarization by protecting TENT5C from the action of miR-4668-5p in rheumatoid arthritis [75]. In line with a role in innate immunity, TENT5C is induced during macrophage activation, and its double KO, together with TENT5A, affects the stability of various mRNAs encoding for secreted immune factors [6]. Notably, Madsen et al. have recently demonstrated how TENT5C silencing in human pancreatic beta and rat insulinoma cell lines results in a significant decrease in insulin release [72]. Finally, TENT5C was recently described as a negative regulator of lentiviral particle production, probably due to its inhibition of autophagy [90]. Altogether, the scientific literature highlights that TENT5C is not only a promising molecular target in different cancers, but also a key regulator of the secretory capacity in many physiological and pathological conditions.

5 | TENT5D/FAM46D

TENT5D is the most tissue-specific member of the family since its expression is reported to be limited to testis. Strengthening the connection with sexual development, TENT5D locus is on chromosome X (Xq21.1). The gene consists of six exons and undergoes alternative splicing, producing two different transcripts encoding the same 389-residue protein with a slightly different 5′-UTR. When compared with the other TENT5s, its N-terminal tail is almost absent and the C-terminal is less conserved. TENT5D was shown to bear the highest affinity for FNDC3 proteins, which mediate its preferential localization at the ER [9]. In line, its overexpression in multiple myeloma cells shows the greatest effects, among the TENT5s, on immunoglobulin production and secretion [9]. At the same time, TENT5D displays the lowest protein stability since it is rapidly degraded by the Ubiquitin-proteasome system [9].

In human TENT5D, two missense (P34L and D42V) and two nonsense mutations (E213* and E275*) have been identified in sterile male affected by oligoasthenoteratozoospermia (OAT) [16–18]. These loss-of-function mutations in the X chromosome result in the absence of TENT5D protein in sperm cells, leading to a lower count and aberrant morphology. Two TENT5D-mutated mouse models reproduced the human phenotype

characterized by structural abnormalities in the heads and the flagella of sperm cells [16, 17]. Moreover, Brouze et al. showed that TENT5D KO mice have a block during the meiosis of sperm cells leading to infertility [12]. Altogether, these results confirm that TENT5D is crucial in spermiogenesis, probably through its ability to stabilize crucial factors like RNASET2, Adam26a, and Cldn34c4 [12, 16].

TENT5D has also been found overexpressed in the cerebral cortex of a mouse model of autism and in patients with Fragile X syndrome who also met the diagnostic criteria for autism [91]. A clear role in cancer of TENT5D is not established, even if it has been reported to be a potential marker for testis tumors [92].

6 | Conclusions and Open Questions

Recent discoveries on noncanonical TENT5 poly(A)polymerases are shedding light on a crucial molecular mechanism, non-nuclear polyadenylation, regulating the stability of mRNAS encoding proteins directed to the secretory apparatus. Thanks to this PAP activity, key roles of TENT5 proteins have been described in cancer, fertility, bone homeostasis, stemness, and immunity.

However, some aspects are still unclear and need more investigation. First, while the other members are clearly associated with a physiological function in a specific cell type or tissue, the role of TENT5B is still controversial. TENT5B expression in secretory cells has negligible effects on protein secretion, suggesting a possible function in a different cellular compartment [9]. Recent work proposed a role of this member in regulating the stability of stemness-related mRNAs [43]. This observation may explain the lack of a specific human pathology associated with TENT5B and prompt further analyses on ncPAP in embryos and stem cells. Furthermore, as described above, the oncosuppressive role of TENT5C in multiple myeloma and its contribution to other cancers is still debated. Recently, Lai et al. proposed a three-way scheme explaining the cellular activities of TENT5C described until now: (1) its role as ncPAP, (2) the inhibitory effect on PLK4 modulating centrosome formation, and (3) the modulation of autophagy and secretion [70]. Further experiments, with different mutants and in vivo models of different cancers, are needed to clarify the multifaceted and complex aspects of this peculiar tumor suppressor. Finally, only one drug, NCTD, has been shown to modulate TENT5 expression and activity [76]. The identification of novel pharmacological modulators of these secretory tuners may provide new tools to manipulate crucial biological processes and will pave the way to new therapeutic strategies against TENT5-associated diseases.

Author Contributions

Daniel Lacidogna, Sara Pennacchio, and Enrico Milan wrote and revised the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All the data are included in the manuscript.

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