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Review article

TRA2: The dominant power of alternative splicing in tumors

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ABSTRACT

The dysregulation of alternative splicing (AS) is frequently found in cancer and considered as key markers for cancer progression and therapy. Transformer 2 (TRA2), a nuclear RNA binding protein, consists of transformer 2 alpha homolog (TRA2A) and transformer 2 beta homolog (TRA2B), and plays a role in the regulation of pre-mRNA splicing. Growing evidence has been provided that TRA2A and TRA2B are dysregulated in several types of tumors, and participate in the regulation of proliferation, migration, invasion, and chemotherapy resistance in cancer cells through alteration of AS of cancer-related genes. In this review, we highlight the role of TRA2 in tumorigenesis and metastasis, and discuss potential molecular mechanisms how TRA2 influences tumorigenesis and metastasis via controlling AS of pre-mRNA. We propose that TRA2Ais a novel biomarker and therapeutic target for cancer progression and therapy.

1. Introduction

According to the latest statistics on cancer, the global incidence of malignant cancer continues to rise and remains the most significant health threat [1,2]. Although multiple factors are recognized to contribute to the occurrence and development of cancer, abnormal regulation of gene expression is one of the key factors in carcinogenicity. Alternative splicing (AS) of messenger RNAs is a fundamental process in eukaryotic gene expression at post-transcriptional level, which enable a gene to form distinct mRNA transcripts, thereby generating diverse proteins. Growing evidence indicates that the dysregulation of AS is frequently found in cancer and considered as a novel signature for cancer progression and therapy. Thus, more and more attention has been attracted to explore the potential regulatory mechanism of AS in tumor biology.

The transformer 2 (TRA2) protein, a nuclear RNA binding protein, was first discovered in *Drosophila melanogaster*. TRA2 proteins are conserved throughout the animal kingdom, and contains two human homologues proteins, transformer 2 alpha homolog (TRA2A) and transformer 2 beta homolog (TRA2B)·In broad terms, TRA2 protein belongs to the serine/arginine-rich (SR) protein family, and generally functions as a critical splicing regulator for mRNA splicing, especially controlling AS of pre-mRNA. Accumulating evidence has suggested that TRA2A and TRA2B are dysregulated in several types of tumors, and participate in the regulation of proliferation, migration, invasion, and chemotherapy resistance in cancer cells through alteration of AS of cancer-related genes.

In this review, we highlight the role of TRA2 in tumorigenesis and metastasis, and particularly discuss potential molecular

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mechanisms how TRA2 influences tumorigenesis and metastasis via controlling AS of pre-mRNA.

1.1. The TRA2A protein structure

In terms of its composition, TRA2 contains two RS (arginine/serine-rich) structural domains on either side of an RNA recognition motif (RRM). TRA2 protein is RNA-binding protein that is involved in splicing regulation, which has been conserved throughout the animal kingdom. Human AS(Alternative splicing)factor *TRA2* has two separate gene paralogs encoding TRA2A and TRA2B proteins。

TRA2A and *TRA2B* are human homologues of the Drosophila splicing regulator *TRA2*, where *TRA2A* is structurally similar to *TRA2B*. *TRA2A* is located on human chromosome 7, while *TRA2B* is located on human chromosome 3. These two proteins share 75% sequence identity and 43% sequence identity with Drosophila TRA2 (Fig. 1). The RNA binding domains and downstream regions of the three proteins have also demonstrated the highest degree of sequence homology. Within this region, 85% of the human structural field is identical to Drosophila TRA2, with 54% similar to the human structural environment [3]. This may explain the similarity between human and Drosophila proteins in terms of their specificity for RNA binding. The main differences between human and Drosophila proteins are the position of the polyglucan extension of the *C*-terminus RS domain as well as the first 49 amino acids of the RS domain at the *N*-terminus. Moreover, all three proteins contain a copy of the tyrosine repeat in the *C*-terminal domain. The lowest sequence homology between Drosophila and human TRA2 has been observed in the RS domain at the *N*-terminus.

TRA2 is a protein that contains both serine and arginine residues near its *N*- and *C*-termini [4]. Consequently, TRA2 belongs to the more prominent SR protein family of the RS domain protein family. The SR protein family serves as a critical factor in alternative splicing [5,6]. An essential characteristic of this protein is its diversity of dipeptide repeats that are rich in arginine and serine (RS domain) [7]. In addition, protein-protein interactions have been described to be mediated by RS domains [8], which are usually phosphorylated *in vivo* [9]. Each SR (Serine-arginine protein) family member performs the same function and is able to complement the splicing of short cytoplasmic extract S100 (all constitutive splicing essential factors other than SR proteins are included in S100). Accordingly, it could splice precursor mRNA during in vitro splicing experiments. TRA2 proteins are distinguishable in function from SR proteins in that they cannot complement the cytoplasmic extract S100 to join precursor mRNA. Therefore, constitutive splicing is not frequently necessary but plays a crucial role in determining splice sites in alternative splicing.

In addition to the RS domain family proteins, TRA2 proteins possess a unique modular structure that calls the RS1 and RS2 domains at their *N*- and *C*-termini. Despite the presence of two RS domains in the TRA2 protein, they serve different functions. Humans possess a more significant RS1 than RS2. In human cells [10], the RS1 domain plays a critical role in splicing and regulation. In contrast, in Drosophila [9], RS2 is essential for controlling the splicing patterns of bisexual genes (genes that produce female and female-specific transcription factors). The RS1 and RS2 domains of the Drosophila RS1 and RS2 proteins have also been shown to differentially activate RNA splicing in a study in which Tra2-MS2 fusion protein targeted RNA via MS2 binding sites consistent with the functionally inequivalent RS structural domains [11].

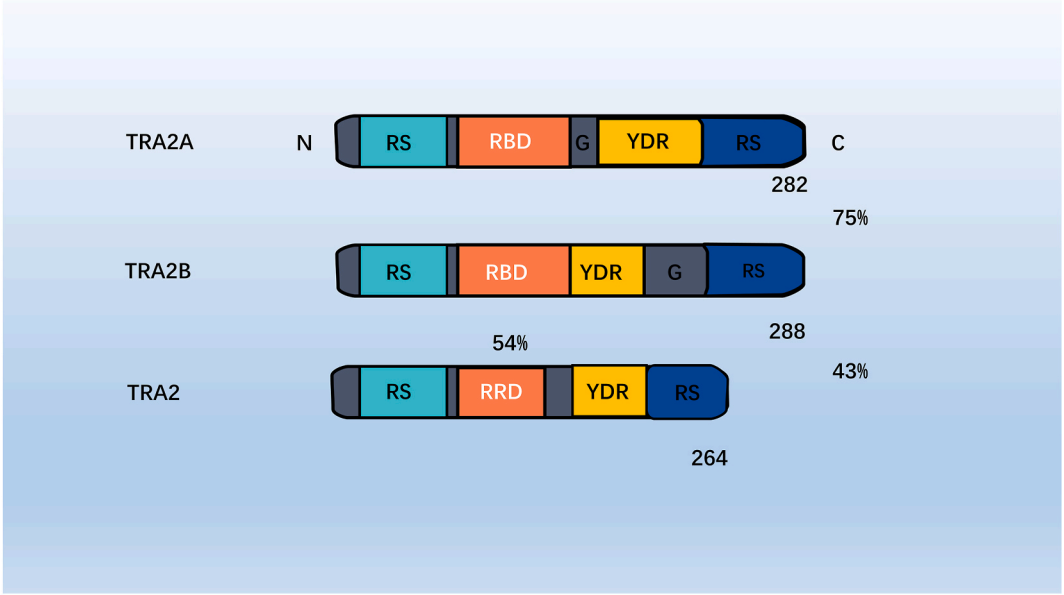

Fig. 1. TRA2A and TRA2B share 75% sequence identity and 43% sequence identity with Drosophila TRA2.

2. Biological role of the TRA2Aprotein

2.1. Functions of the TRA2 protein

TRA2 proteins play a crucial role in the development and physiology of organisms.

In regard to functionality, the TRA2 protein is necessary in order to determine the sex of a female and plays a role in spermatogenesis in Drosophila [12,13]. It is possible to partially restore sex determination in female TRA2-deficient flies by expressing human TRA2A; however, this does not replace the endogenous TRA2 protein in all regulatory splicing processes. This suggests that in both invertebrate and vertebrate TRA2 homologues [14], there is substantial functional protection between them, which echoes the structural differences between human TRA2A and TRA2 proteins. TRA2B has been shown to play a critical role in controlling mouse embryonic development in knockout gene experiments [15]. Embryos with TRA2B knockouts have been found to exhibit embryonic disorders of embryonic day 7.5 (E7.5) and death during the early stages of development. In the nervous system, conditional deletion of TRA2B has been described to impair normal brain development [16–18]. Moreover, cortical TRA2B knockout mice (conditional knockout driven by EMX-Cre in the brain) have been shown to be able to survive to adulthood, though they developed severe brain abnormalities due to apoptosis in cortical neural progeny. However, TRA2B knockout mice with a broader range of neurons (a conditional knockout driven by Nestin) have died early after birth, demonstrating severe cerebral cortex and thalamic abnormalities.

Physiologically, TRA2B affects smooth muscle cell properties via alternative splicing in order to regulate the targeting subunit of myocardin phosphatase [19], which is also targeted by cardiotonic steroid digitalis glycosides [20]. The expression of TRA2 protein is regulated by the DNA topoisomerase inhibitor camptothecin [21]. A recent study has found that the simultaneous deletion of TRA2A and TRA2B led to substantial changes in splicing [22], suggesting that TRA2A and TRA2B are functionally redundant. TRA2B exerts an inhibitory solid feedback effect on TRA2A, which buffers splicing changes when one of the proteins is missing. Following deletion of either TRA2A or TRA2B, splicing defects have been shown to be minor but statistically significant, suggesting that TRA2A and TRA2B are interconvertible. Accordingly, it may be critical for the entire organism as well as the specific developmental points (e.g., brain or testis development) to control splicing profiles by splicing feedback control of the combined TRA2 protein concentration, as splicing feedback control may finely affect splicing profiles. After TRA2B is exhausted, TRA2A replaces TRA2B functionally and essentially maintains TRA2 target exons containing the TRA2 protein, which may be necessary to include the constitutive exon. When depletion of both TRA2 protein levels reduced cell viability, it was concluded that the simultaneous deletion of TRA2A and TRA2B, rather than a single deletion, resulted in significant changes in the splicing of endogenous TRA2B target exons. In addition, it has been shown that both constituent target exons and alternative target exons are produced under the control of double TRA2A-TRA2B. Through parallel compensation, TRA2 proteins have been found to collectively control constitutive and alternative splicing patterns in order to maintain cell viability [22]. In actuality, both purified proteins preferentially bind RNA sequences that contain GAA repeats, which is a characteristic of many enhancer elements. Neither TRA2 protein functions constitutively spliced in vitro, though both have been shown to bind in a specific sequence-binding manner to activate enhancer-dependent splicing and recover upon inhibition of competitive RNA. This suggests that human TRA2 protein, which is a sequence-specific splicing activator, may be involved in cell-specific regulation.

2.2. TRA2 with alternative splicing

The TRA2 protein has been shown to be involved in the selective splicing of various genes [3,11,23,24]. For example, pre-mRNA splicing regulates several vital processes in vertebrates and invertebrates, including the development of the mouse brain and the determination of sex in Drosophila. Genes are widely transcribed before leaving the nucleus in order to form the human gene encoding precursor messenger RNA (pre-mRNA), which includes splicing exons and introns prior to developing into mature mRNA, which usually occurs at high fidelity to produce functional mRNA. RNA splicing is comprised of constitutive splicing and alternative splicing. Alternative splicing refers to synthesizing precursor mRNA (pre-mRNA) to synthesize proteins with different structures and functions [25,26]. Approximately 95% of transcription can be synthesized from other proteins from one mRNA through alternative splicing [11]. The splicing of constitutive exons enters all mRNA transcribed by a gene. Notably, alternatively spliced exons may sometimes be included or excluded. Human protein-coding genes generate three mRNA isoforms via alternative splicing, with nearly all varying in regulation.

Moreover, the different variants formed by alternative splicing are subject to various developmental and tissue-specific regulations [27–32]. Studies have shown that alternative splicing occurs with different forms of tumorigenesis and drug resistance [33–38]. Alternative splicing and its associated factors are deregulated in cancer. Every cancer signature, including chemoresistance, is associated with splicing switching, enabling it to exhibit a more aggressive cancer phenotype [39,40].

As a splicing activator, TRA2 is closely associated with splicing regulation. TRA2 often functions as a splicing activator by binding to ESE (exon-splicing enhancers). TRA2 proteins bind to ESE sequences close to regulatory splicing sites in order to increase utilization of their respective regulatory splicing sites and strengthen spliceosome assembly [41]. The TRA2 protein activates the cassette exon-intron, which is the most common alternatively spliced form in both human and mouse cells. The presence of multiple TRA2 binding sites in the target RNA is characteristic of several TRA2 protein regulatory exons. The requirement of multiple binding sites can be applied to models of TRA2 protein function. There is a weak 5-splice site in the human testis-specific HIPK3-T exon. HIPK3-T includes four ESEs activated by TRA2B, which work together in order to initiate the soft 5-splice site in response to increased concentrations of TRA2B [10]. TRA2B activation has been shown to be completely blocked by removing TRA2B-responsive ESE from the HIPK3-T exons. Thus, the four TRA2B-reactive ESE may compensate for the weak 5-splicing of the HIPK3-T exons by providing a threshold for enhanced activity.

TRA2 activation has been found to activate the weak 3 splice site, and one of the most well-studied splicing targets of TRA2 is the bisexual gene in Drosophila. Initially, TRA2 proteins were discovered in light of their crucial role in sex selection in Drosophila, though they also function in sex selection in other species of insect [42–44]. TRA2 has been shown to activate alternative 5 splicing sites. Fruitless genes are known to encode different transcription factor isoforms essential for female and male development. This fruitless transcription factor regulates the result of a male-specific muscle called Lawrence's muscle, which is vital for normal sexual behavior in male animals [45]. It is known that fruitless alternative splicing of mRNA can form both male and female spliced mRNA isoforms. The fruitless precursor mRNA produces both male and female altered mRNA isoforms 45. In the fruitless gene, TRA2-reactive ESE is very close to the weak splice site of female 5 (within 38 nucleotides) and includes three copies of the CA-rich TRA2 binding site. Upon binding to Tra and TRA2, ESE led to the selection of a weak spot.

In preventive medicine, human TRA2A determines influenza A virus-host adaptation by regulating viral mRNA splicing [46]. Human TRA2A (huTRA2A) activates IAV replication by modulating viral messenger RNA (mRNA) splicing. Moreover, huTRA2 has been shown to suppress mRNA splicing by binding to intron splicing silencing motifs of representative avian MmRNA/H5N1 or NSmRNA viruses. Consequently, human and avian viruses replicate differently in vitro and vice versa. This phenomenon confirmed that sites M − 334 and NS-234/236 are essential for TRA2A [47]binding, mRNA splicing, viral replication, and pathogenicity. This study suggests rational strategies for protecting public health by understanding how the avian influenza virus adapts to the human host.

2.3. TRA2 and non-coding RNA

Non-coding RNA are a class of RNA that is characterized by their lack of protein translation. A DNA sequence that transcribes ncRNAs is called a non-coding RNA gene. The present understanding of ncRNAs continues to evolve with the development of biological studies as well as the influence of other disciplines. Recently, several new ncRNAs have been identified. Although they do not encode proteins, they have been shown to participate in the protein translation process and serve as critical molecules in RNA. These include RNA with various known sequences, such as rRNA, tRNA, snRNA, microRNA, and RNA with unknown parts. These non-coding RNA can be divided into three categories [47–49]:less than 50 nt, including microRNA, siRNA, and piRNA; 50 nt to 500 nt, including rRNA, tRNA, snRNA, snoRNA, SLRNA, and SRP RNA; and more than 500 nt, including long mRNA-like and long non-coding RNA without a polyA tail.

By performing a literature review, TRA2A was found to possess many connections with lncRNAs and miRNAs, which play a vital role in its stability. Considerable research has shown that the TRA2A/LINC00662/ELK4 axis regulates blood-brain barrier permeability in the Alzheimer's disease (AD) microenvironment [50]. Additionally, TRA2A and LINC00662 have been found to be enriched in microvascular endothelial cells (ECs) in an in vitro blood-brain barrier model cultured with Aβ1-42. TRA2A increases LINC00662 stability by binding to it. LINC00662 degrades ELK4mRNA through SMD (Staufen1-mediated mRNA decay) and downregulates ELK4mRNA, which then downregulates ELK4 by binding to ZO-1, occludin, and claudin-5 promoters. Therefore, it further reduces blood-brain barrier permeability in the AD microenvironment, resulting in further aggravation of AD. As BBB permeability is regulated by the TRA2A, LINC00662, and ELK4 axes, this application may serve as a novel therapeutic target for treating Alzheimer's disease.

Various scholars have proposed that in ncRNA m6a modification, TRA2A functions as a regulator [51]. Accordingly, TRA2A may

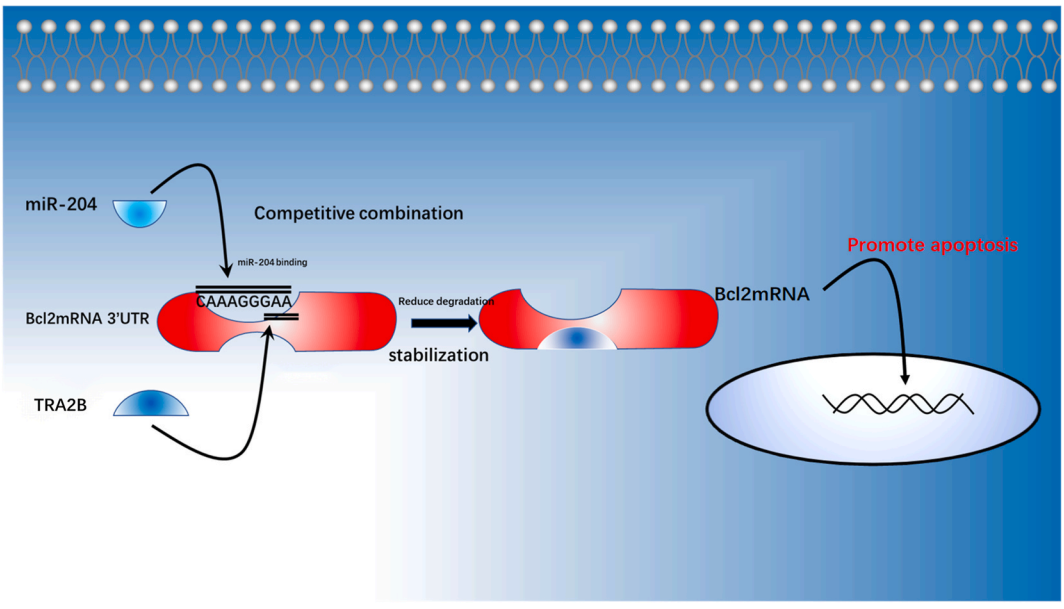

Fig. 2. TRA2B and miR-204 overlap with partial binding sites of Bcl2mRNA.

selectively promote the methylation of the m6A site coexisting with the binding site on MALAT1 (metastasis-associated in lung adenocarcinoma transcript 1) by interacting with m6a. This methylation results in an altered MALAT1 structure that promotes PRC2/EZH2 histone methylase complex formation. However, further research should be conducted in order to ascertain its specific regulatory mechanisms.

TRA2B and miRNAs are two essential factors in post-transcriptional regulation that can coordinate target mRNA translation and stability and produce antagonistic effects [52]. By employing RNA binding protein immunoprecipitation RIP and microarray analysis of the potential target mRNA, Bcl2 mRNA was shown to be a potential target in regulating apoptosis. Furthermore, the TRA2B protein and miR-204 overlap with partial binding sites of Bcl2mRNA (Fig. 2); therefore, they can regulate Bcl2 protein-mediated apoptosis signaling through a combination of both competition as well as the non-coding region of the 3 ends of Bcl2α mRNA [53]. Furthermore, TRA2A is directly bound to pre-mRNAs rich in AGAA or RAAG and stimulates variable exons in mRNA, causing its isoform transformation, which results in a reduction of protein levels. Silent *TRA2B* gene expression in human colon cancer cells have been shown to downregulate Bcl2 mRNA and protein expression by accelerating Bcl2α and Bcl2β degradation. However, the splicing pattern of Bcl2 and gene promoter activity have not been shown to be altered [54].

3. Relationship between TRA2A protein and tumors

3.1. Role of TRA2A protein in tumors

TRA2A is a TRA2 (transformer 2) homologous family member, which is a protein with an RRM (RNA recognition motif) domain. TRA2A is mainly localized to the nucleolus, *TRA2A* is located on human chromosome 7. Moreover, restriction to vesicles, high or moderate expression levels have been detected in 7 of the 78 standard tissue cell types that have been analyzed. The renal tubules, smooth muscle cells, subsets of cells in the testicular seminiferous tubules, and exocrine cell fraction in the pancreas have demonstrated strong cytoplasmic positivity. Intense cytoplasmic and membrane staining has also been observed in cardiomyocytes. Superficial squamous epithelium has been found to demonstrate marked membrane staining, while nuclear immunoreactivity has been observed in neuronal cells and megakaryocytes. The remaining normal cells are considered to be harmful. Antibody staining is present in 6% of cancers detected by cell staining. Here, endometrial cancer, thyroid cancer, lymphoma, malignant melanoma, renal cancer, and pancreatic cancer exhibit intense positive immunohistochemical staining for nucleoli. The tumor parts of certain colorectal and endometrial cancers have also demonstrated moderately positive TRA2A immunohistochemically stained nucleoli.

The meta-analysis of the gene profiling data has shown that abnormal expression of *TRA2A* in endometrial cancer, thyroid cancer, lymphoma, malignant melanoma, and kidney cancer, as well as the upregulation of *TRA2A* in liver cancer [55], are closely related. *TRA2A* has consistent chromosomal changes and has been shown to be in a dysregulated methylation pattern in childhood pineal germ cell tumors. Furthermore, gliomas have been described to be overexpressed during neuronal processes [56]. Patients who have glioma and are undergoing chemotherapy have exhibited *TRA2A* somatic mutations according to whole-exome sequencing [57]. *TRA2A* is highly expressed in SHG44 cells and promotes proliferation, migration, invasion, and epithelial cell-to-mesenchymal transformation [58].

In triple-negative breast cancer (TNBC), microarray analysis has shown that targets are rich in cancer-related functions. Moreover, the expression of proliferation markers, such as *PCNA, CDC25A*, and *CDC6*, are increased following overexpression of *TRA2A*, which promotes the proliferation, invasion, migration, and survival of TNBC cells. The aforementioned studies have found that *TRA2A* controls the alternative splicing of *MELK, CALU, RSRC2, CEACAM1, LMCD1, PALM,* and *RFWD2*, resulting in a more aggressive phenotype of *TRA2A* -overexpressing tumor cells. In addition, following paclitaxel (PTX) treatment, significant isoforms of CALU, RSRC2, and PALM have been found in surviving cancer cells. Researchers have also shown that RSRC2 (arginine/serine-rich coiled-coil 2) is regulated from RSRC2s to RSRC2l [59]. This alternative splicing process promotes paclitaxel (PTX) resistance, in which RSRC2 has been identified as a tumor suppressor associated with chemosensitivity [39]. *TRA2A*, which is considered to be independent of other splicing factors, enhances PTX resistance in TNBC via specific modulation of cancer-related or tumor suppressor-associated splicing and may serve as a prognostic indicator of breast cancer. The TRA2A -RSRC2s-RSRC21 splicing pathway is targeted in order to deregulate TRA2A and RSRC2 expression and alleviate tumor cell resistance to PTX. This finding may help provide a novel target for subsequent drug development and pave the way for new modalities of breast cancer treatment.

Recent studies have demonstrated that four candidate genes (*MGMT, TRA2A, RPS6KA2, and U2AF1*) serve as biological features of prostate cancer in the DU-145, PC-3, and L NCAP prostate cancer cell lines as well as specimen biopsies [60]. Many cell functions rely on *TRA2A*, including DNA damage repair, mRNA splicing, the mitogen-activated protein kinase 6 (MAPK) pathway, and JMJD3 demethylase. Its inhibitor GSK-J4 regulates *MGMT, TRA2A, RPS6KA2,* and *U2AF1* genes in prostate cancer cell lines, which may provide new avenues for research in the development of novel therapeutics.

In terms of esophageal cancer, 194 esophageal samples were downloaded and analyzed from The Cancer Genome Atlas (TCGA) (https://www.cancer.gov/aboutnci/organization/ccg/research/structural-genomics/tcga) in this study, in which 10 samples were normal, 95 were ESCC (esophageal squamous carcinoma), and 89 were EAC (esophagi adenocarcinoma). Different types of genetic alterations were present in approximately 12% of the tumor models, including amplification (12 cases), deep deletion (1 point), and mRNA upregulation (12 points). There was a statistically significant association between patients with *TRA2A* mutations and less than five years of disease-free survival ($P = 0.006$) and overall survival ($P = 0.016$), suggesting that *TRA2A* mutations are associated with prognostic factors in cancer, which may have certain clinical prognostic relevance. In esophagi squamous SCC and adenocarcinomas, TRA2A stimulates both proliferation and migration [50] and binds to specific sites on MALAT1 (2341-4500bp fragments). It has been speculated that the interaction of TRA2A with MALAT1 may improve the stability of lncRNAs in esophagi squamous EC cells.

Long-chain, non-coding MALAT1 is a nuclear lncRNA that has been confirmed to be involved in various cancer lymphatic invasions, distant metastasis, and tumor differentiation nuclei [61,62]. It is a multifunctional regulator that activates downstream genes through multiple molecular mechanisms and promotes the induction of migration and transfer (Fig. 3). MALAT1 is known to sponge miRNA (miR-101 and miR-217), causing the miRNA-target mRNA [63] to decrease. Moreover, mir101 is a regulator of EZH2, which significantly affects the downside targets of miRNAs and lncRNAs. Furthermore, MALAT1 recruits EZH2 to form the PRC2 complex, while increased Ezh2 expression could upregulate β-catenin and promote the β-catenin/Wnt signaling pathway [63]. Ectopic expression or deletion of TRA2A changes the MALAT and EZH2/β-catenin pathway. *TRA2A* acts as a typical function splicing mRNA factor and participates in EZH2/β-catenin transduction pathways by directly binding lncRNA as an RNA binding protein, which acts as a lncRNA stabilizer, thus providing a new pathway in regard to the mechanism and treatment of cancer.

Whether the alternative splicing of TRA2A leads to cancer and whether there is an unknown pathway have both been speculated, which should be investigated further as it may be popular in future cancer research.

3.2. Relationship between TRA2B protein and tumors

TRA2B is a member of the TRA2 (transformer 2) homologous family, which consists of 10 exons and nine introns and produces five splice isoforms (TRA2B1 to TRA2B5) [64]. TRA2B has an AGAA-rich sequence as its primary RNA-binding site [65–67]. While the AGAA RNA sequence is effective against TRA2, the NGAA sequence is adequate. In addition, by replacing the first A nucleotide in the AGAA target sequence with a C, G, or T nucleotide, the binding efficiency reduces (Kd values increase 2-fold between AGAA and NGAA). Second, TRA2B is capable of switching to a different RNA-binding mode, namely, the stem-loop structure [67]. CAA-rich single-stranded sequence interactions have been observed in the analysis of 79 standard tissue cell types, in which high or moderate expression levels were detected in 78 samples. TRA2B has also been shown to be strongly positive for nucleolar staining by immunohistochemistry. Moreover, cytoplasmic and membrane staining has been observed in a few cases. Humans with age-related macular degeneration have been shown to experience upregulation and aberrant [49] localization of the TRA2B protein in the nucleus of retinal cells along with differential expression [68]. In addition to being differentially expressed in certain cancers, TRA2B has been linked to other diseases, such as spinal muscular atrophy (SMA) [69], Alzheimer's disease [70], and FTDP-17 [71] (frontotemporal dementia and Parkinson's disease).

TRA2B is essential during embryonic development, and TRA2B proteins are involved in many embryonic development pathways in cell growth and motility. TRA2B splicing targets identified in normal tissues are crucial in cancer cell biology, particularly in cell division, movement, and invasion [18]. Usually, it is deactivated in normal cells but is frequently reactivated in cancerous cells. Previous studies have demonstrated that TRA2B is associated with cerebral ischemia [72], nerve damage [73], Alzheimer's disease [70]and other disorders. TRA2B regulates the alternative splicing of numerous genes in a concentration-dependent manner, including calcitonin/calcitonin gene-associated peptide (CGRP), myosin phosphatase (MYPT1), surviving motor neurons (SMN), and microtubule-associated protein tau (TAU) [69,71,74,75]. When *TRA2B* levels increase in cancer cells, it suggests that the *TRA2B* may be able to bypass the standard feedback control mechanisms. This is a crucial feedback control mechanism that utilizes TRA2A and has an alternatively spliced toxic exon. When poison exons are spliced into mRNA, they introduce premature stop codes that prevent the translation of the full-length protein and often target the mRNA [76] in order to conduct a nonsense-mediated decay. The toxin exon splicing process for TRA2B mRNA is activated when it binds to TRA2B. The splicing of this toxin exon then allows for increased TRA2B

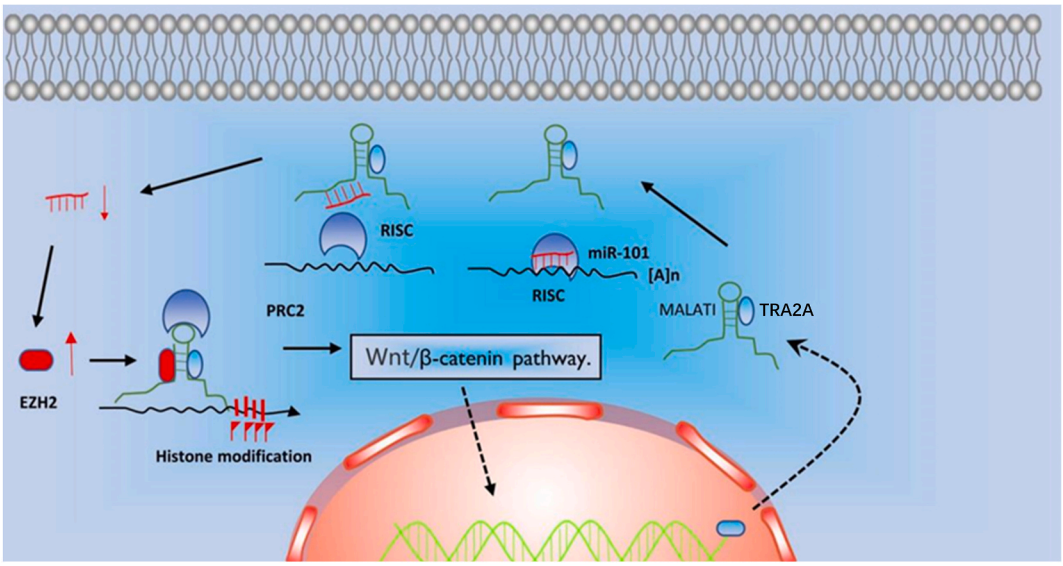

Fig. 3. TRA2A binds with MALAT1; MALAT1 also recruits EZH2 to form the PRC2 complex, promoting the β-Catenin/Wnt signaling pathway.

protein production. Increased expression of the TRA2B protein should cause an increase in TRA2B toxic exon intrusions; in turn, through the negative feedback loop [77], it reduces the newly translated TRA2B protein.

TRA2B is recognized as an oncogene and is upregulated in invasive breast cancer compared to other invasive cancers [78]. Progression and metastasis are closely related. The TRA2B protein plays a specific role in recognizing variable exons in CD44 cells. Increased expression induces CD44 exon v4 and v5 inclusions while promoting further enhanced invasiveness and metastatic ability of CD44 cells [79]. In gastric cancer, the downregulation of *TRA2B* inhibits the growth of gastric cancer cell lines, as measured by the corresponding reduction in BrdU incorporation, which monitors entry into the S phase of cells [80]. Knockdown of *TRA2B* in colon cancer cells reduces cell viability and increases apoptosis, as demonstrated by the TUNEL assay of PARP levels [81]. These findings provide new insights into the development of colon cancer. *TRA2B* levels have also been shown to be higher in high-grade malignant gliomas compared to lower-grade gliomas, where *TRA2B* downregulation inhibited cell proliferation and migration [82]. *TRA2B* overexpression is a significant predictor of relapse and poor survival in human prostate cancer [83]. In lung cancer, interactions between miR-335 and TRA2B have been found to regulate the proliferation of cancer cells [84]. TRA2B promotes the proliferation, invasion, and migration of laryngeal SCC cells by activating the PI3K/AKT signaling pathway in laryngeal carcinoma. Scholars have also proposed that TRA2B is related to cancer cell survival and drug sensitivity [53,85]. Thus, TRA2B significantly contributes to the development and progression of multiple cancers.

In addition to being amplified by the *TRA2B* gene, the *TRA2B* gene also serves as a transcriptional target of the proto-oncogene ETS-1, which may lead to higher levels of transcription in cancer cells expressing this transcription factor. ETS-1, which is encoded by the proto-oncogene, affects metastasis, proliferation, and cell survival. The increased expression of transcription factor ETS-1 may account for the upregulation of *TRA2B* in cancer cells. For example, in metastatic breast cancer, ETS-1 expression has been shown to be associated with poor prognosis and an aggressive phenotype [86,87]. In human colon cells, *TRA2B* gene transcription has been observed to be regulated by combining transcription factors HSF1 and ETS-1 as well as through the positive regulation of their proximal promoter regions [80]. Furthermore, ETS-1 and *TRA2B* [88] expression may also be controlled by estrogen [86], which acts as the critical factor that drives the progression of estrogen receptor-positive breast cancer. These findings suggest that changes in transcription factors may cause the pathological mechanism of *TRA2B* upregulation in cancer cells.

ROS generation of reactive oxygen species during inflammation may illustrate the potential mechanism pertaining to the upregulation of *TRA2B* in cancer cells. In rat brains, *TRA2B* expression has been shown to be activated following astrocyte hypoxia [89]. Moreover, *TRA2B* expression in smooth muscle cells has been similarly induced upon reoxygenation of hypoxic cells [90]. *TRA2B* has also been found to be upregulated in the oxidative stress response of the human colorectal cancer cell line HCT116 [80]. Ischemia has also been described to cause accumulation of *TRA2B* in the cytoplasm, which is accompanied by changes in splicing sites [72]. However, whether TRA2B can be used as an indicator for cancer prognosis, a popular area in clinical cancer research, requires further exploration".

4. Outlook

According to the domain of each TRA2 member, its *C*-terminal sequence, and their specific functions, TRA2 family proteins may be better understood in the future based on functional changes in mutant cells with the *C*-terminal exchange/deletion of the TRA2 protein. Although why the *TRA2* is upregulated in tumors remains unclear, a global analysis has shown that each TRA2 protein plays a different role in oncogenic signaling. Accordingly, future studies may target the unique function of each TRA2 protein and develop new strategies for targeted therapies.

Studies of *TRA2* in leukemia, such as prostate cancer, gastric cancer, and liver cancer, have also been reported; however, their detailed mechanisms require further investigation. As a core member of the RS protein family, TRA2 is highly expressed in various common malignancies and diseases and is closely related to the biological behavior and prognosis of tumor occurrence, development, invasion, and metastasis while belonging to a specific type of oncogene. Therefore, it serves as a potential target in the study of novel antitumor treatment. Further clarification of how *TRA2* promotes tumor development could provide a theoretical basis for tumorigenesis and clinical applications.

The inclusion of an exon during splicing is dependent on the binding of splicing factors to short low-complexity regulatory sequences. The exon selection process depends on RNA-binding proteins or splicing factors, which enhance or inhibit exon inclusion and follow two primary principles. First, splicing factors bind to short introns or exon motifs (or splicing regulatory sequences), which are usually low-complexity lines consisting of the same nucleotide or dinucleotide repeats. Furthermore, the interaction of joining factors with their cognate-binding motifs generally depends on the sequence context as well as the presence of clusters of associated binding motifs. Second, the splicing outcome (i.e., exon inclusions or jumps) depends on the location of the splicing factor relative to the regulated exon binding to the pre-mRNA. For example, HNRNP-like splicing factors generally inhibit the inclusion of exons that they bind to. However, they naturally enhance exon inclusion when insecure and are instead introns. The interaction between TRA2A and TRA2B as the most critical transduction protein has rarely been studied, which would be analyzed in our subsequent study. The unique functions of each TRA2 protein will also be explored in the future, and novel strategies will be developed in order to treat the conserved and unique oncogenicity of targeted TRA2 proteins.

Cancer is associated with many features [91], including the ability for cells to divide, escape growth inhibitors by maintaining proliferation signaling, resist cell death, induce angiogenesis to ensure oxygen and nutrient supply, and invade other parts of the body (metastasis). Such features occur during other changes, including reduced genomic stability and inflammation.

Changes in splicing patterns in cancer cells compared to normal cells can contribute to the occurrence of these features by influencing the expression patterns of important protein subtypes that regulate cell behavior. Splicing alterations in cancer cells are

partly the result of the core spliceosome, specific components, and RNA-binding proteins that regulate selective exon intrusions [92], thereby changing activity and expression. Alterations in the splicing environment of cancer cells may also confer therapeutic effects, and drugs targeting the spliceosome are currently in development for the treatment of cancer [93–97].

Author contributions

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Ethical compliance

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Data access statement

Data will be made available on request.

Declaration of competing interest

No potential conflicts of interest were disclosed.

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