

## Review Article

# Expression of Tra2 $\beta$ in Cancer Cells as a Potential Contributory Factor to Neoplasia and Metastasis

Andrew Best,<sup>1</sup> Caroline Dagniesh,<sup>1</sup> Ingrid Ehrmann,<sup>1</sup> Mahsa Kheirollahi-Kouhestani,<sup>1</sup> Alison Tyson-Capper,<sup>2</sup> and David J. Elliott<sup>1</sup>

<sup>1</sup> Institute of Genetic Medicine, Newcastle University, Central Parkway, Newcastle upon Tyne NE1 3BZ, UK

<sup>2</sup> Institute of Cellular Medicine, Newcastle University, Framlington Place, Newcastle upon Tyne NE2 4HH, UK

Correspondence should be addressed to David J. Elliott; david.elliott@ncl.ac.uk

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The splicing regulator proteins SRSF1 (also known as ASF/SF2) and SRSF3 (also known as SRP20) belong to the SR family of proteins and can be upregulated in cancer. The *SRSF1* gene itself is amplified in some cancer cells, and cancer-associated changes in the expression of *MYC* also increase *SRSF1* gene expression. Increased concentrations of SRSF1 protein promote prooncogenic splicing patterns of a number of key regulators of cell growth. Here, we review the evidence that upregulation of the SR-related Tra2 $\beta$  protein might have a similar role in cancer cells. The *TRA2B* gene encoding Tra2 $\beta$  is amplified in particular tumours including those of the lung, ovary, cervix, stomach, head, and neck. Both *TRA2B* RNA and Tra2 $\beta$  protein levels are upregulated in breast, cervical, ovarian, and colon cancer, and Tra2 $\beta$  expression is associated with cancer cell survival. The *TRA2B* gene is a transcriptional target of the protooncogene ETS-1 which might cause higher levels of expression in some cancer cells which express this transcription factor. Known Tra2 $\beta$  splicing targets have important roles in cancer cells, where they affect metastasis, proliferation, and cell survival. Tra2 $\beta$  protein is also known to interact directly with the RBMY protein which is implicated in liver cancer.

## 1. Introduction

Cancer is associated with a number of distinctive disease hallmarks [1]. These hallmarks include the ability of cancer cells to continuously divide by maintaining proliferative signalling pathways and to evade growth suppressors, to resist cell death; to induce angiogenesis to ensure a supply of oxygen and nutrition, and to invade other parts of the body (metastasis). These hallmarks of cancer cells occur against other changes including decreasing genome stability and inflammation [1].

Changes in splicing patterns in cancer cells compared to normal cells can contribute to each of these cancer hallmarks through effects on the expression patterns of important protein isoforms which regulate cell behaviour [2–4]. The splicing alterations which occur in cancer cells are partially due to changes in the activity and expression of core spliceosome components [5] and in the RNA binding proteins which regulate alternative exon inclusion [6]. Changes in the splicing environment in cancer cells might have therapeutic

implications. Drugs which target the spliceosome are also being developed as potential therapies for treating cancer patients [7].

In this review, we particularly examine the potential role of the splicing regulator Tra2 $\beta$  as a modulator of gene function in cancer cells. Tra2 $\beta$  is part of a larger protein family which contains RNA recognition motifs (RRMs) and extended regions of serine and arginine residues (RS domains, named following the standard 1 letter amino acid code for serine and arginine) [8–10]. Core SR proteins include SRSF1 (previously known as ASF/SF2) and SRSF3 (previously known as SRP20) (Figure 1). Tra2 $\beta$  is considered an SR-like protein rather than a core SR family member because of two features. Firstly, Tra2 $\beta$  contains both an N- and C-terminal RS domains (each of the core members of the SR family has just a single C-terminal RS domain, with the RRM at the N-terminus). Secondly, the core group of SR proteins but not Tra2 $\beta$  can restore splicing activity to S100 extracts [11]. S100 extracts are made from lysed HeLa cells by high-speed

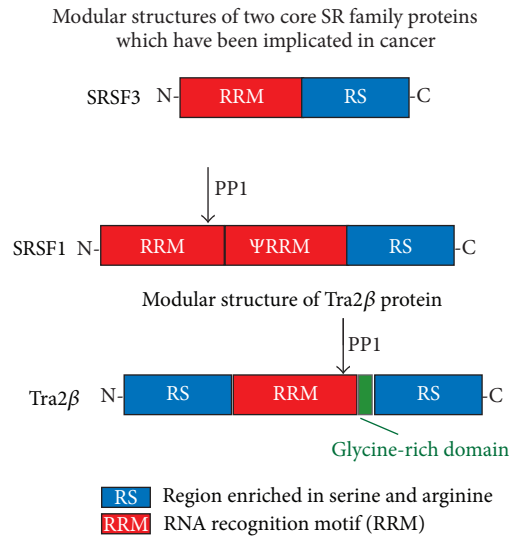


FIGURE 1: Modular structure of the core SR family proteins SRSF1 (also known as ASF/SF2) and SRSF3 (also known as SRp20) and the SR-like protein Tra2β. The RNA recognition motif (RRM) binds to target RNAs, and the RS region is responsible for protein-protein interactions. SRSF1 has a second RRM, annotated ψRRM. SRSF1 and Tra2β have a PP1 docking site.

ultracentrifugation to remove nuclei but contain most of the core spliceosome components necessary for splicing with the important exception of SR proteins which are insoluble in the magnesium concentrations used [12]. Addition of any single SR protein is sufficient to restore splicing activity to these S100 extracts [13].

Tra2β protein functions as a splicing regulator in the cell nucleus, where it activates the inclusion of alternative exons [14, 15]. Tra2β protein is able to interact with two types of RNA targets through its RRM. Firstly, the major RNA binding site for Tra2β is an AGAA-rich sequence [11, 16, 17]. Although an AGAA RNA sequence works best for Tra2β protein, an NGAA sequence is actually sufficient for binding. However, substituting the first A with either C, G, or T nucleotides in the NGAA target sequence decreases binding efficiency (the Kd value increases 2-fold between AGAA and NGAA) [16]. Secondly, the RRM of Tra2β is able to switch to a second mode of RNA binding, in which it interacts with single-stranded CAA-rich sequences within a stem loop structure [17].

When Tra2β binds to target RNA sites within an exon, it activates splicing inclusion of these bound exons into mRNA [11, 15–17]. Splicing activation by Tra2β protein is concentration dependent: increased Tra2β protein concentration leads to increased levels of target exon splicing inclusion [14, 15]. The RRMs of Tra2β and SRSF1 proteins both contain a docking site for protein phosphatase 1 (PP1), and dephosphorylation of these proteins by PP1 affects alternative splicing regulation [18].

Tra2β protein is encoded by the *TRA2B* gene (also called *SFRS10*) on human chromosome 3. As well as any potential role in cancer cells, Tra2β has important roles in normal

development and is essential for normal mouse embryonic and brain development (*TRA2B* knockout mice fail to develop normally) [15, 19]. *TRA2B* has a paralog gene called *TRA2A* on the long arm of human chromosome 7, and this paralog encodes Tra2α protein [20]. Paralogs are additional copies of a gene derived by duplication. *TRA2A* derived by gene duplication from *TRA2B* early in the vertebrate lineage and so is found in all vertebrates.

A number of the SR proteins have been found to have roles in cancer, amongst them, SRSF1 and SRSF3 (Figures 1 and 2). The mechanism of SRSF1 upregulation in cancer cells has been explained at a mechanistic level, and the effects of this upregulation in terms of gene expression control have been mapped onto the pathway of oncogenesis. Here, we review these important principles for SRSF1 and then apply these principles to gauge the likely effect of the Tra2β protein on cancer-specific gene expression.

## 2. SRSF1 Is Upregulated in Cancer and Is a Target for the Prooncogenic Transcription Factor Myc

SRSF1 upregulation in cancer cells can occur through two distinct mechanisms. Firstly, the *SRSF1* gene itself can become amplified in cancer. The *SRSF1* gene is on a region of chromosome 17q23 which is amplified in some breast cancers, including in tumours with a poor prognostic outlook and in the MCF7 breast cancer cell line [21]. Analysis of the *SRSF1* gene on the cBio Cancer Genomics Portal shows amplification of *SRSF1* mainly in breast cancers (Figure 2) [22, 23]. Secondly, *SRSF1* gene transcription is activated by the prooncogenic transcription factor Myc which is itself activated in some cancers. Myc upregulation in cancer leads to downstream increases in both *SRSF1* mRNA and SRSF1 protein expression [24].

Protein expression analysis using a highly specific monoclonal antibody showed that a number of tumours have increased SRSF1 protein compared to normal tissue [21]. As well as being upregulated in some cancer cells, *SRSF1* operates as a bona fide oncogene. Increased *SRSF1* gene expression can transform rodent fibroblasts in an NIH3T3 assay, and the resulting transformed cells form tumours in nude mice [21]. Tumour formation by these transformed fibroblasts is directly dependent on *SRSF1* expression, since it is blocked by parallel shRNA inhibition of *SRSF1* [21]. Together, these data suggest that upregulation of *SRSF1* gene expression can be one of the initial steps in oncogenesis.

Experiments support an important function for SRSF1 protein in breast cancer cells. Mouse COMMA1-D mammary epithelial cells form tumours more efficiently in mice after transduction with *SRSF1*, and transduction of MF10A cells with *SRSF1* results in increased acinar size and decreased apoptosis in a 3D culture model [25]. A number of splicing targets have been identified which respond to increased levels of *SFRS1* expression in cancer cells (Table 1). These SRSF1-driven splicing changes produce prooncogenic mRNA splice isoforms, which encode proteins which decrease apoptosis and increase cellular survival and proliferation.

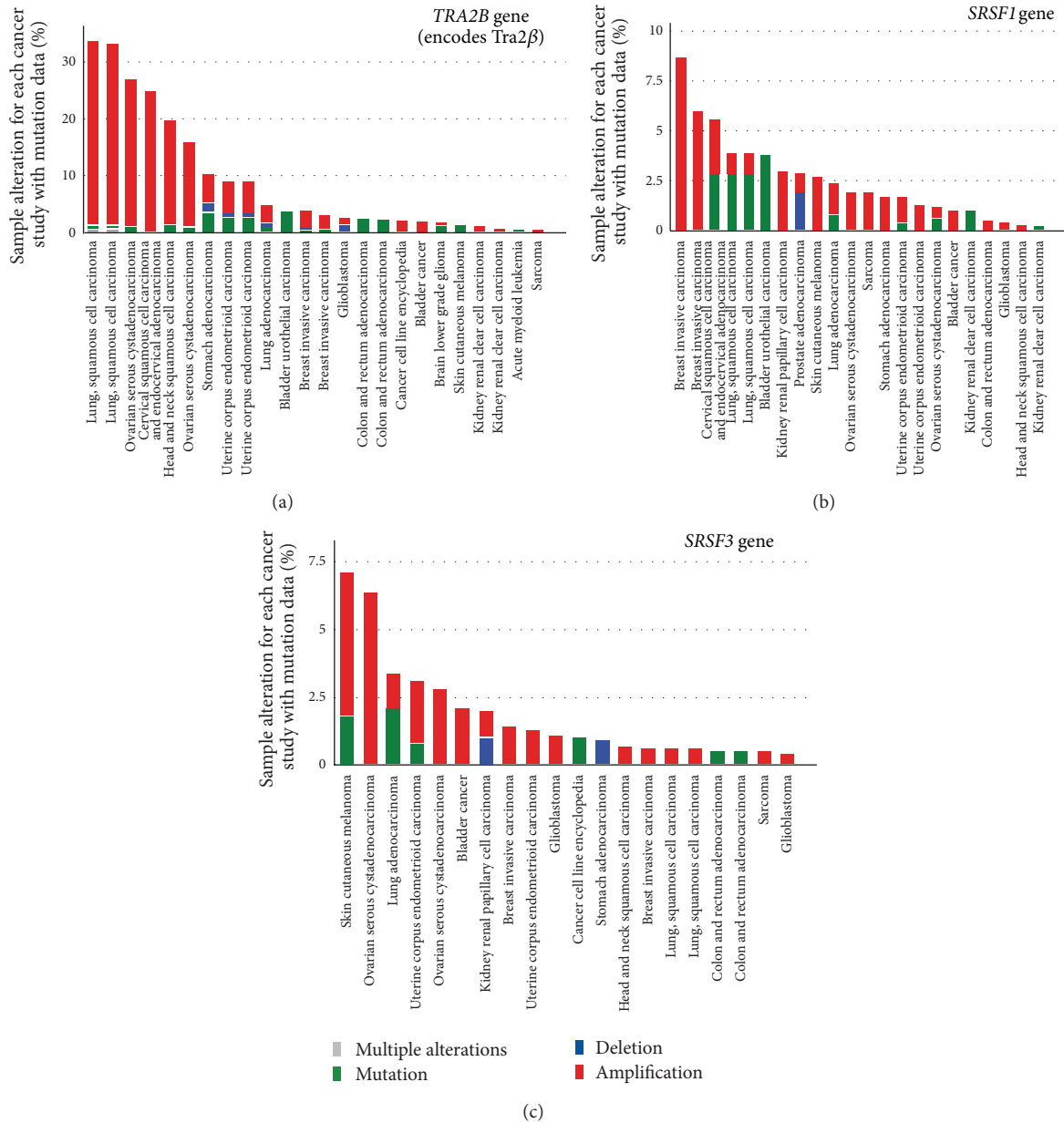


FIGURE 2: The (a) *TRA2B*, (b) *SRSF1* (also known as ASF/SF2), and (c) *SRSF3* (also known as SRP20) genes are amplified or otherwise mutated in several cancer types. For each of the three genes, data for genetic changes in all cancers were obtained using the cBioPortal database, filtering for percentage of altered cases (studies using mutation data) [22, 23]. The percentage, of cancer samples which showed genetic alterations in large cancer studies are shown on the Y axis and the respective type of cancer on the X axis. Full details of this kind of analysis are given on the cBioPortal website <http://www.cbioportal.org/public-portal/index.do>.

### 3. Increased *SRSF3* Expression Is Also Associated with Cancer

Increased expression of the SR protein *SRSF3* is also associated with cancer. The *SRSF3* gene is amplified in some cancers (Figure 2) [22, 23]. Loss of *SRSF3* expression in a number of cancer cell lines increases apoptosis and decreases proliferation, and increased expression of *SRSF3* leads to transformation of rodent fibroblasts and enables them to form tumours in nude mice [26].

Increased *SRSF3* expression levels have been associated with an increased tumour grade in ovarian cancer [27]. Intracellular levels of *SRSF3* mRNA are important for cancer cells: siRNA-mediated downregulation of *SRSF3* leads to cell cycle arrest at G1 in colon cancer cells, and their increased death through apoptosis. The mechanism of increased apoptosis in response to higher levels of *SRSF3* protein might include aberrant splicing of the *HIPK2* pre-mRNA (which encodes an important apoptotic regulator related to *HIPK3*, which is a known splicing target of Tra2 $\beta$ ), such that

TABLE 1: Known prooncogenic splicing targets of SRSF1 (previously known as ASF/SF2).

Splicing target	Possible role in cancer cells	Reference
<i>RON</i>	$\Delta$ exon11 splice isoform increases cell motility and metastasis	[21, 25]
<i>BIN</i>	<i>BIN12a</i> splice isoform encodes protein no longer able to bind Myc and acts as tumour suppressor	[21, 25]
<i>MNK2</i>	<i>MNK2</i> 13b splice isoform makes kinase which can phosphorylate EIF4E independent of MAP kinase activation	[21, 25]
<i>S6K</i>	Promotes oncogenic isoform of S6kinase which phosphorylates small subunit of ribosome	[21, 25]
<i>MCL-1/BCL-X/CASPASE9</i>	Promotes production of antiapoptotic mRNAs to result in cell survival	[63–65]

a proteasome-resistant form of HIPK2 protein is made after SRSF3 depletion [28].

#### 4. *Tra2 $\beta$* Is Amplified in Particular Cancers and Is a Target of the Oncogenic Transcription Factor ETS-1

The *TRA2B* gene which encodes *Tra2 $\beta$*  becomes amplified in several cancers (Figure 2) and particularly in cancers of the lung, cervix, head and neck, ovary, stomach, and uterus [22, 23]. Upregulation of *Tra2 $\beta$*  protein expression has also been observed in several cancers, including breast, cervical and ovarian [29–31], and colon [32]. *Tra2 $\beta$*  upregulation is associated with invasive breast cancer [30], and medium to high *Tra2 $\beta$*  expression correlates with a poorer prognosis in cervical cancer compared to patients with lower expression levels [29].

*Tra2 $\beta$*  protein expression has been demonstrated to be important for cancer cell biology. Downregulation of *Tra2 $\beta$*  inhibits cell growth of a gastric cancer cell line, measured by a corresponding decrease in BrdU incorporation which monitors cells which have entered S phase [33]. Knockdown of *Tra2 $\beta$*  in colon cancer cells reduced cell viability and increased the level of apoptosis monitored using a TUNEL assay and through measurement of levels of cleaved PARP [32].

As well as *TRA2B* gene amplification, the expression levels of the ETS-1 transcription factor provide a possible mechanism through which *Tra2 $\beta$*  might be upregulated in cancer cells. Regulated transcription of the *TRA2B* gene in human colon cells is positively controlled by binding of the HSF1 and ETS-1 transcription factors to its promoter proximal region [32]. The ETS-1 protein is itself encoded by a protooncogene. ETS1 expression in metastatic breast cancer correlates with a poor prognosis [34, 35] and is associated with an invasive phenotype [36]. Expression of both ETS-1 [35] and *Tra2 $\beta$*  [37] might also be under control of estrogen,

which is a key driver of estrogen receptor positive breast cancer development. Taken together, these observations suggest that the pathological mechanism of *Tra2 $\beta$*  upregulation in cancer cells might result from underlying changes in transcription factors in cancer cells. Other positive regulators of cell growth might also stimulate *Tra2 $\beta$*  expression, since expression of *Tra2 $\beta$*  is upregulated in response to growth factors in normal smooth muscle cells [38].

Reactive oxygen species made during inflammation provide a further potential mechanism for *Tra2 $\beta$*  upregulation in cancer cells. *Tra2 $\beta$*  expression is activated in response to reoxygenation of astrocytes following a period of oxygen deprivation and by ischaemia in rat brains [39]. Expression of *Tra2 $\beta$*  in smooth muscle cells is similarly induced following reoxygenation of hypoxic cells [38], and is upregulated in response to oxidative stress in human colorectal carcinoma cell line HCT116 [32]. Ischaemia has also been reported to induce cytoplasmic accumulation of *Tra2 $\beta$*  along with accompanying changes in splice site use [40]. *Tra2 $\beta$*  translocates into the cytoplasm in gastric cancer cells in response to cell stress induced by sodium arsenate [32], and changes in the nuclear concentration of *Tra2 $\beta$*  might have downstream effects on the splicing inclusion of target exons.

The increased levels of *Tra2 $\beta$*  observed in cancer cells mean that the *TRA2B* gene must be able to bypass the normal feedback expression control mechanisms which exist to keep *Tra2 $\beta$*  protein levels under tight control. An important feedback control mechanism uses an alternatively spliced “poison exon” in the *TRA2B* gene. Poison exons introduce premature stop codons when they are spliced into mRNAs, preventing translation of full-length proteins and often targeting mRNAs for nonsense-mediated decay [41]. Poison exon splicing into the *TRA2B* mRNA is activated by binding of *Tra2 $\beta$*  itself. Splicing inclusion of this poison exon acts as a brake on production of more *Tra2 $\beta$*  protein. The predicted outcome is that increased expression of *Tra2 $\beta$*  protein should lead to increased *TRA2B* poison exon inclusion and so correspondingly less newly translated *Tra2 $\beta$*  protein through a negative feedback loop [42].

Similarly, the levels of SRSF1 and the other SR proteins are thought to be normally autoregulated through poison exon inclusion [43]; so these other SR proteins must similarly bypass these mechanisms in cancer cells to enable their higher levels of expression to be established.

#### 5. *Tra2 $\beta$* Protein Regulates Splicing Patterns Which Are Important to Cancer Cells

How might upregulation of *Tra2 $\beta$*  affect the biology of cancer cells? Three *Tra2 $\beta$* -target exons have been identified in genes known to have important roles in cancer cells (Table 2). For two of these target exons, the actual regulated isoforms have also been demonstrated in cancer cells.

Firstly, strong *Tra2 $\beta$*  binding to a cancer-associated exon in the *nuclear autoantigenic sperm protein* (abbreviated *NASP*) gene has been detected using HITS-CLIP of endogenous *Tra2 $\beta$*  protein in the mouse testis [14, 15]. This *Tra2 $\beta$* -target exon is abbreviated *NASP-T*. Whilst the somatic

TABLE 2: Known pro-oncogenic splicing targets of Tra2 $\beta$ .

Splicing target	Possible role in cancer cells	Reference
<i>CD44</i>	Affects cancer cell mobility and metastasis	[30]
<i>Homeodomain-interacting kinase 3 (HipK3)</i>	<i>HIPK3</i> increases phosphorylation of cJun and cell proliferation	[57]
<i>Nasp-T</i>	Histone chaperone important for efficient replication Implicated in DNA repair processes	[15]

*NASP* splice isoform is expressed ubiquitously, the *NASP-T* splicing isoform has a much tighter anatomic distribution and its splicing is associated particularly with cancer cells and embryonic development. While most normal adult tissues do not splice the *NASP-T* exons into their mRNAs, high levels of splicing inclusion are seen in the testis and to a lesser extent the heart, gut, and ovary [15].

Splicing inclusion of the *NASP-T* exon is strongly activated in transfected cells in response to coexpression of Tra2 $\beta$ , and *NASP-T* splicing also decreases in *TRA2B* knockout mouse brains compared to wild type, confirming that the *NASP-T* exon is a bona fide regulated target exon of Tra2 $\beta$  [14, 15]. Tra2 $\beta$  is currently the only known splicing regulator of the *NASP-T* exon. The *NASP-T* exon is unusually long (a 975 nucleotide long cassette exon, while the typical size for a human exon is more like 120 nucleotides), with at least 37 Tra2 $\beta$  protein binding sites within its sequence, making a very responsive target for Tra2 $\beta$  expression. Splicing inclusion of the *NASP-T* exon into the *NASP* mRNA introduces the coding information for an extra 375 amino acids into the encoded *NASP* protein (Figure 3).

The *NASP* protein has a strongly biased peptide sequence which contains a high frequency of glutamic acid residues. The negative charges of the glutamic acid residues facilitate interactions with the positively charged histone partner proteins that *NASP* protein interacts with. *NASP* proteins also use tetratricopeptide repeats (TPRs) and histone binding motifs to facilitate interactions with protein partners including histones [44]. Both the somatic (s*NASP*) and *NASP-T* isoforms of the *NASP* protein contain the same TPRs involved in protein-protein interactions and seem to be functionally interchangeable in cells [45]. However, the longer *NASP-T* protein isoform has an additional histone binding motif and a longer stretch of the glutamic-acid-enriched sequence, suggesting that it might more efficiently interact with histones (Figure 3(a)). The *NASP-T* peptide cassette also adds a number of potentially phosphorylated serine and threonine residues to the *NASP* protein [44, 46]. Splicing inclusion of the *NASP-T* exon is likely to be important in cancer cells. The specific siRNA-mediated downregulation of *NASP* mRNAs containing the *NASP-T* exon leads to a block in proliferation and increased levels of apoptosis in cancer cells [47, 48].

Isoforms of the *NASP* protein with and without the peptide cassette inserted by the *NASP-T* exon are molecular

chaperones which import histone H1 into the nucleus [49]. *NASP* protein isoforms also stably maintain the soluble pools of H3 and H4 histones needed for assembly of chromatin at times of high replication activity and are part of the complexes which load these into chromatin [45]. The *NASP* gene is critical for cell cycle progression in cultured cells and for mouse embryogenesis [50].

Why might *NASP* protein be important for cancer cells? *NASP* belongs to a network of genes important for cell survival [51], and *NASP* protein is a tumour-associated antigen in ovarian cancer [52]. *NASP* is highly expressed in S phase of the cell cycle [49], when chromatin needs to be reassembled after replication. Higher levels of *NASP* protein expression might be needed by cancer cells to enable their higher rates of replication to be achieved. *NASP* protein also has other roles related to chromatin stability. *NASP* protein is phosphorylated by the ATM and ATR kinases in response to ionising radiation and implicated in the repair of DNA double strand breaks [53]. One of the protein partners of *NASP* protein is the DNA repair protein Ku, and the yeast homologue of *NASP* is present at double strand breaks suggesting an important role in DNA repair (reviewed in [44]).

The second known splicing target of Tra2 $\beta$  with likely important functions in cancer cells is within the *CD44* pre-mRNA. *CD44* encodes an important transmembrane protein partly displayed on the cell surface as the CD44 antigen (Figure 3(b)). CD44 protein acts as a receptor for hyaluronic acid and possibly other molecules and controls interactions with other cells, the extracellular matrix, and cellular motility through modulation of intracellular signalling cascades [54].

The N- and C-termini of the CD44 protein are encoded by constitutive exons, but the *CD44* gene also contains an internal block of 10 consecutive internal alternative exons which are differentially regulated during development and in cancer [55]. These alternative exons encode portions of the extracellular domain of the protein (Figure 3(b)). *CD44* variable exons show variant splicing inclusion in breast cancer cells [30]. In particular, two *CD44* internal variable exons, *CD44v4* and *CD44v5*, increase their splicing inclusion in transfected HeLa cells in response to increased Tra2 $\beta$  protein expression [30], suggesting that Tra2 $\beta$  might also increase their inclusion in breast tumours with elevated Tra2 $\beta$  expression. Although expression of variant CD44 exons has historically been associated with cancer metastasis, the picture regarding *CD44* alternative splicing in cancer is complex. Very recent data suggest that the standard isoform of *CD44* mRNA (without splicing inclusion of its variable exons) might in fact play a key role in metastatic breast cancer, particularly in enabling an epithelial-mesenchyme transition of breast cancer cells [56].

The third known Tra2 $\beta$ -target exon which might be potentially relevant in cancer cells is in the *HIPK3* gene, which encodes a serine/threonine kinase involved in transcriptional regulation and negative control of apoptosis. High cellular levels of Tra2 $\beta$  stimulate splicing inclusion of a poison exon called HIPK3-T into the *HIPK3* mRNA [57]. Normal *HIPK3* protein is concentrated in subnuclear structures called promyelocytic leukemia bodies (PML bodies). The shorter

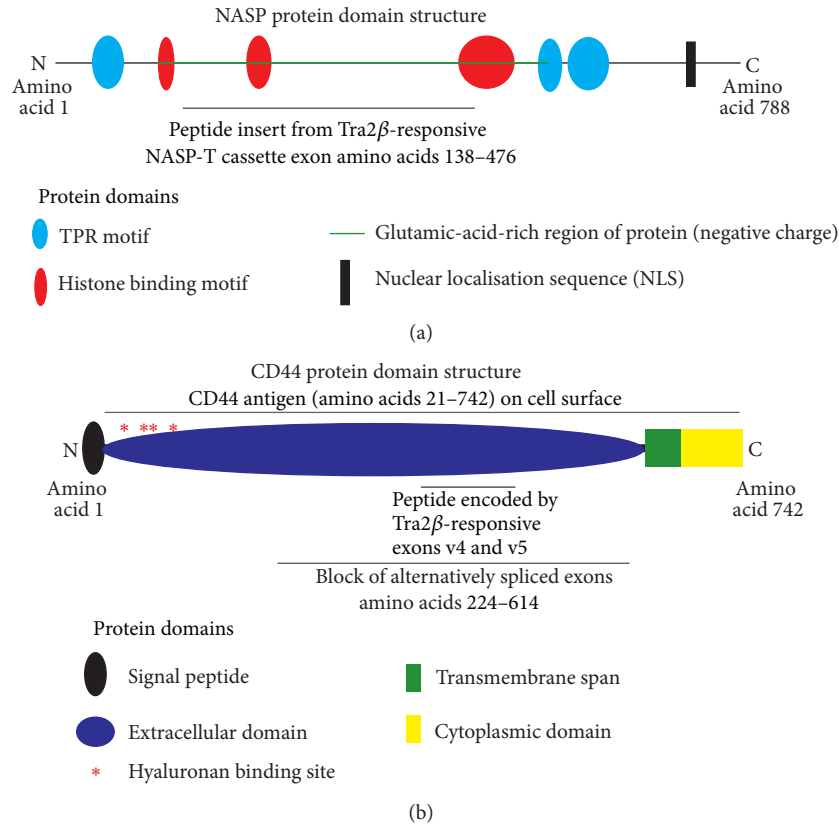


FIGURE 3: Protein domain architecture of known *Tra2β* splicing targets which are expressed in cancer cells. (a) Modular structure of NASP protein assembled from the UniProt database (<http://www.uniprot.org/uniprot/P49321>) [46], showing the position of the peptide insert encoded by the *Tra2β*-target exon NASP-T. (b) Modular structure of CD44 protein assembled using information from the UniProt database (<http://www.uniprot.org/uniprot/P16070#P16070-6>) [46], showing the position of peptide sequences encoded by the *Tra2β*-target exons CD44 v4 and v5. The CD44 antigen is displayed on the cell surface, and the protein is anchored on the cell surface by a single trans-membrane domain. Alternative isoforms are made through alternative splicing of 10 exons out of 19 encoding amino acids in the extracellular domain and also 2 exons which encode peptide sequence in the cytoplasmic domain. The two exons reported CD44 v4 and v5 exons correspond to amino acids 386–428 and 429–472, respectively, in the encoded protein. The protein domain structures are not drawn to scale.

HIPK3 protein isoform made under control of *Tra2β* fails to localise in PML bodies and lacks regions of the protein predicted to bind the androgen receptor, homeodomains, Fas, and p53 [57]. HIPK3-T is not confirmed as a splicing target of *Tra2β* in cancer, since splicing of the HIPK3-T exon has only been observed thus far in human testis and has not been directly reported from cancer cells [57].

## 6. *Tra2β* Is Involved in Protein Interaction Networks with Partner Proteins Involved in Cancer

Some of the proteins which are known to interact either directly or indirectly with *Tra2β* have themselves been implicated with roles in cancer cells. *Tra2β* directly interacts with members of the hnRNP G family of proteins which includes the prototypic member hnRNP G (encoded by the *RBMX* gene located on the X chromosome); RBMY protein (which is encoded by a multigene family on the Y chromosome); and a number of retrogene-derived proteins.

Of these retrogene-derived proteins, one called HNRNP G-T is both highly conserved in mammals and specifically expressed in meiosis. The interaction between *Tra2β* and hnRNP G family members likely buffers the splicing activity of *Tra2β* [58, 59], although they might also coregulate some target exons [60]. Expression of the RBMY protein has been directly implicated in liver cancer biology, where it may contribute to the male specificity of this cancer [61]. RBMY protein also interacts with SRSF3 protein [62].

## 7. Summary

The splicing regulator *Tra2β* is upregulated in some human cancers. Possible mechanisms for this upregulation include changes in oncogenic transcription factor expression and oxygen free radical concentrations in neoplastic tissue, both of which affect *TRA2B* gene expression (Figure 4). We do not currently know whether the *TRA2B* gene can function as an oncogene in its own right until experiments to test transformation of NIH3T3 cells are performed or the behaviour of such transformed cells in nude mice is tested. However, we

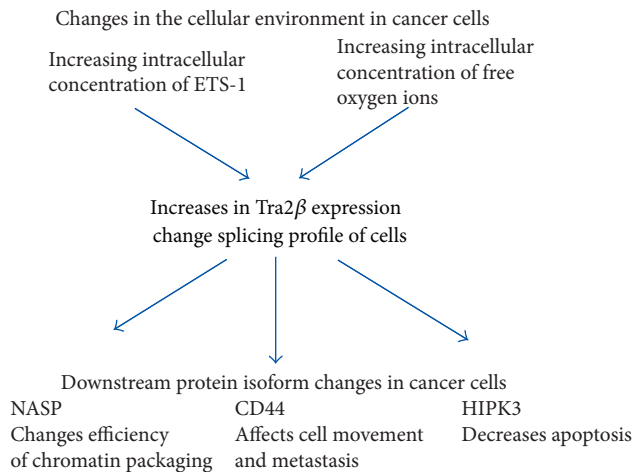


FIGURE 4: Hypothetical model suggesting how changes in the cellular environment may influence the expression of Tra2 $\beta$  and lead to downstream changes in mRNA splice isoform production.

do know that some of the known splicing targets of Tra2 $\beta$  identified in normal tissues are important for cancer cell biology and are particularly implicated in cell division and motility. Tra2 $\beta$  is essential during embryonic development, and many embryonic developmental pathways involved in cell growth and motility which are turned off in adult cells often become reactivated in cancer cells. Future analysis of the role of Tra2 $\beta$  in cancer cells will require the detailed identification of its endogenous splicing targets in cancer cells and the elucidation of their physiological roles.

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