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

Andrew Best, 1 Caroline Dagliesh, 1 Ingrid Ehrmann, 1 Mahsa Kheirollahi-Kouhestani, 1 Alison Tyson-Capper, 2 and David J. Elliott 1

1 Institute of Genetic Medicine, Newcastle University, Central Parkway, Newcastle upon Tyne NE1 3BZ, UK
2 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].
Modular structures of two core SR family proteins which have been implicated in cancer

SRSF3

RRM | RS | C

PP1

SRSF1

RRM | ψRRM | RS | C

Modular structure of Tra2β protein

Tra2β

RS | RRM | RS | C

PP1

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 Tra2x 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 SRSF1 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.
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 Tra2β), such that
Table 1: Known prooncogenic splicing targets of SRSF1 (previously known as ASF/SF2).

<table>
<thead>
<tr>
<th>Splicing target</th>
<th>Possible role in cancer cells</th>
<th>Reference</th>
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<tbody>
<tr>
<td>RON</td>
<td>Δ exon11 splice isoform increases cell motility and metastasis [21, 25]</td>
<td></td>
</tr>
<tr>
<td>BIN</td>
<td>protein no longer able to bind Myc and acts as tumour suppressor [21, 25]</td>
<td></td>
</tr>
<tr>
<td>MNK2</td>
<td>EIF4E independent of MAP kinase activation [21, 25]</td>
<td></td>
</tr>
<tr>
<td>S6K</td>
<td>Promotes oncogenic isoform of S6 kinase which phosphorylates small subunit of ribosome [21, 25]</td>
<td></td>
</tr>
<tr>
<td>MCL-1//BCL-X/CASPASE9</td>
<td>Promotes production of antiapoptotic mRNAs to result in cell survival [63–65]</td>
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a proteasome-resistant form of HIPK2 protein is made after SRSF3 depletion [28].

4. Tra2β Is Amplified in Particular Cancers and Is a Target of the Oncogenic Transcription Factor ETS-1

The *TRA2B* gene which encodes Tra2β becomes amplified in several cancers (Figure 2) and particularly in cancers of the lung, cervix, head and neck, ovary, stomach, and uterus [22, 23]. Uregulation of Tra2β protein expression has also been observed in several cancers, including breast, cervical and ovarian [29–31], and colon [32]. Tra2β upregulation is associated with invasive breast cancer [30], and medium to high Tra2β expression correlates with a poorer prognosis in cervical cancer compared to patients with lower expression levels [29].

Tra2β protein expression has been demonstrated to be important for cancer cell biology. Downregulation of Tra2β 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 Tra2β 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 Tra2β might be upregulated in cancer cells. Regulated transcription of the *TRA2B* gene in human colon cells is positively controlled by binding of the HSFI 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 Tra2β [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 Tra2β upregulation in cancer cells might result from underlying changes in transcription factors in cancer cells. Other positive regulators of cell growth might also stimulate Tra2β expression, since expression of Tra2β 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 Tra2β upregulation in cancer cells. Tra2β expression is activated in response to reoxygenation of astrocytes following a period of oxygen deprivation and by ischaemia in rat brains [39]. Expression of Tra2β 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 Tra2β along with accompanying changes in splice site use [40]. Tra2β 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 Tra2β might have downstream effects on the splicing inclusion of target exons.

The increased levels of Tra2β observed in cancer cells mean that the *TRA2B* gene must be able to bypass the normal feedback expression control mechanisms which exist to keep Tra2β 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 Tra2β itself. Splicing inclusion of this poison exon acts as a brake on production of more Tra2β protein. The predicted outcome is that increased expression of Tra2β protein should lead to increased *TRA2B* poison exon inclusion and so correspondingly less newly translated Tra2β 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. Tra2β Protein Regulates Splicing Patterns Which Are Important to Cancer Cells

How might upregulation of Tra2β affect the biology of cancer cells? Three Tra2β-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 Tra2β binding to a cancer-associated exon in the *nuclear autoantigenic sperm protein* (abbreviated NASP) gene has been detected using HITS-CLIP of endogenous Tra2β protein in the mouse testis [14, 15]. This Tra2β-target exon is abbreviated NASP-T. Whilst the somatic
Table 2: Known pro-oncogenic splicing targets of Tra2β.

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<th>Splicing target</th>
<th>Possible role in cancer cells</th>
<th>Reference</th>
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<tr>
<td>CD44</td>
<td>Affects cancer cell mobility and metastasis</td>
<td>[30]</td>
</tr>
<tr>
<td>Homeodomain-interacting kinase 3 (HipK3)</td>
<td>HIPK3 increases phosphorylation of c-Jun and cell proliferation</td>
<td>[57]</td>
</tr>
<tr>
<td>Nasp-T</td>
<td>Histone chaperone important for efficient replication</td>
<td>[15]</td>
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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 Tra2β, 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 Tra2β [14, 15]. Tra2β 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 Tra2β protein binding sites within its sequence, making a very responsive target for Tra2β 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 tetraarcic peptide repeats (TPRs) and histone binding motifs to facilitate interactions with protein partners including histones [44]. Both the somatic (sNASP) 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 Tra2β 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 Tra2β protein expression [30], suggesting that Tra2β might also increase their inclusion in breast tumours with elevated Tra2β 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 Tra2β-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 Tra2β 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
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 prototypical 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 hnRNPs is likely to modulate the splicing activity of Tra2β [58, 59], although they might also co-regulate 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
Increasing intracellular concentration of ETS-1

Increasing intracellular concentration of free oxygen ions

Increases in Tra2β expression change splicing profile of cells

Downstream protein isoform changes in cancer cells

NASP
Changes efficiency of chromatin packaging

CD44
Affects cell movement

HIPK3
Decreases apoptosis

Figure 4: Hypothetical model suggesting how changes in the cellular environment may influence the expression of Tra2β and lead to downstream changes in mRNA splice isoform production.

do know that some of the known splicing targets of Tra2β identified in normal tissues are important for cancer cell biology and are particularly implicated in cell division and motility. Tra2β 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 Tra2β 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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