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Investigation of BIN1 Isoforms in Cells Expressing a Viral Oncoprotein

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Investigation of BIN1 Isoforms in Cells Expressing a Viral Oncoprotein

By:

Kristen DeRosa

This thesis is submitted in partial fulfillment of the requirements for Honors in the Discipline in Biology and the Elizabethtown College Honors Program

May 1, 2019

Thesis Advisor _____

Second Reader _____

Third Reader _____

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Introduction:

The process of pre-mRNA splicing is known to be a critical step in the regulation of gene expression in various diseases. Splicing is carried out by a ribonucleoprotein complex known as the spliceosome, which associates with a conserved family of splicing regulators known as Serine/Arginine-rich (SR) proteins. These proteins, known as splicing factors, contain both RNA-binding and protein interaction domains and are responsible for selecting specific splice sites in the mRNA (Tazi et al., 2008). Many of these splicing factors have been shown to have a prominent role in tumorigenesis and tumor progression (da Luz et al., 2017). While incorrect expression and deregulation of splicing factors can result in tumor development, alternative splicing regulated by these factors can also modulate the expression of many protooncogenes and tumor-suppressor isoforms (Karni et al., 2007). In humans, over 90% of protein coding genes are alternatively spliced, enabling a single gene to encode multiple mRNA transcripts and therefore multiple protein isoforms (Tazi et al., 2008). This is achieved through the inclusion or exclusion of certain exons or introns. Splice variants of specific genes resulting from alternative splicing events have exhibited significant involvement in cancer cell biology including modulation of both proliferation and apoptosis.

Serine/arginine-rich splicing factor 1 (SRSF1) is a member of the SR protein family known to bind exonic splicing enhancers (ESEs) to promote the recognition of both constitutive and alternative splice sites during spliceosome assembly (Goncalves et al., 2014). Upregulation of SRSF1 has been frequently observed in tumors of the colon, thyroid, kidney, lung, liver, pancreas and breast, likely relating to its alternative splicing of genes associated with tumorigenesis (Das et al., & Karni et al., 2007). Due to its ability to promote the establishment and maintenance of cellular transformation, SRSF1 is deemed an oncoprotein. Further, overexpression of SRSF1 promotes alternative splicing of transcripts involved in apoptosis, including pro-apoptotic proteins BIM and BIN1 in transformed mammary epithelial cells (Anczukow, et al., 2012). This increase in alternative splicing due to SRSF1 upregulation resulted in a lack of apoptosis, thus promoting cell proliferation.

The human BIN1 gene is located on chromosome 2 and encodes the nucleocytoplasmic protein known as bridging integrator 1. In typical cells, BIN1 is expressed ubiquitously and is thought to be involved in multiple mechanisms including clatherin-mediated endocytosis, endosome trafficking and inducing membrane curvature (Dräger et al., 2017). However, in malignant human cells, BIN1 possesses features of a tumor suppressor, as its expression has been shown to lead to apoptosis (DuHadaway et al., 2001). These features are mediated through the ability of BIN1 to physically and functionally interact with the Myc oncoprotein (Wechsler-Reya et al., 1997). This interaction seems to be dependent on a functional Myc binding domain (MBD) in BIN1 (Figure 3A). The MBD, encoded by exons 13 and 14, interacts with the highly conserved Myc Box 1 (MB1) sequence at the N terminus of Myc. (Weschler-Reya et al., 1997 & Pineda-Lucena et al., 2005). The integrity of both MB1 and MB2 have been shown to be essential for its interaction with BIN1 (Sakamuro et al., 1996). The SH3 domain at the C-terminus of BIN1 is also integral in the BIN1-Myc interaction (Pineda-Lucena et al., 2005). In the absence of Myc,

the proliferation rate of transformed cells was not affected by the presence of a BIN1 protein containing the MBD, further confirming the interaction of the two proteins (Elliott et al., 1999).

The Myc oncoprotein, sometimes referred to as a 'master regulator', is a well-known transcription factor which has been studied extensively for its central role in various aspects of cellular growth regulation, including the development and maintenance of metabolic changes that promote cellular transformation (Miller et al., 2012). In fact, it is though that Myc overexpression contributes to the cause of at least 40% of tumors (Miller et al., 2012). While its functions seem to be complex, the direct interaction of the MBD in full length BIN1 with Myc is known to inhibit this Myc-mediated cell proliferation and transformation through multiple mechanisms, including promotion of apoptosis (Elliott et al., 1999). This is supported through the finding that BIN1 is frequently downregulated or inactivated in Myc associated breast and prostate cancers as well as malignant melanoma (DuHadaway et al., 2001).

In addition to the full-length protein with tumor suppressor features, up to 10 tissue specific BIN1 isoforms have been identified (Weschler-Reya et al., 1997). These isoforms are produced due to alternative splicing mediated by SRSF1 and the majority of splicing events occur between exons 9 and 16 (Anczukow et al., 2012). Some of these splice variants, including a transcript which includes exon 12A (BIN1 + 12A, Figure 3B), are tumor associated isoforms and have been shown to lack the pro-apoptotic functions associated with the full length BIN1 (Ge et al., 1999). Overexpression or elevated levels of SRSF1 in transformed and cancerous cells leads to the production of BIN1 + 12A splice variants, suggesting an alteration in the tumor suppressor activity of the protein when exon 12A is present (Anczukow et al., 2012 & Ge et al., 1999). Though the exact function of exon 12A is unknown, it is found to encode a consensus class I SH3-binding motif which interacts with its own SH3 domain (Pineda-Lucena et al., 2005). Specifically, a PxxP motif within exon 12A intramolecularly interacts with the C-terminal SH3 domain and thus competes for binding with c-Myc (Pineda-Lucena et al., 2005). Ultimately, BIN1 + 12A transcripts are unable to bind Myc, cannot induce apoptosis and therefore cannot inhibit Myc-mediated cellular transformation. Studies utilizing BIN1 proteins lacking the entire MBD (BIN1ΔMBD) describe the inability of this protein to inhibit colony formation in neuroblastoma cell lines with Myc amplification (Tajiri et al., 2003). Additionally, BIN1ΔMBD proteins are unable to inhibit endogenous Myc activity (Lundgaard et al, 2011). This further suggests the importance of an intact MBD for BIN1's ability to inhibit the oncogenic properties of Myc.

Interestingly, one of the many genes which Myc targets is the splicing factor SRSF1. Myc is able to bind directly to the SRSF1 promoter and activate its transcription (Goncalves et al., 2015). These two proteins are significantly co-expressed in various cancer types and the Myc induced increase in SRSF1 levels can lead to an increase in alternative splicing of transcripts, including those of BIN1 (Das et al., 2012). The SRSF1 overexpression in some cancers has been shown to promote the production of the BIN1+12A isoform, which is unable to bind Myc and lacks the tumor suppressor activity of the full-length protein (Anczukow et al., 2012). This suggests that Myc and SRSF1 are able to indirectly regulate apoptosis by promoting the production of the BIN1 + 12A isoform, which lacks pro-apoptotic activity. Due to the inability

of BIN1+12A to bind Myc, there will be an increase of free and active Myc in the cancerous cells. This free Myc is then able to activate transcription of more SRSF1, which promotes the production of more BIN1+12A isoforms, thus forming a positive feedback loop resulting in cellular transformation.

While research studies performed with primary tumor cell lines are ideal for the study of cancer biology pathways, the utilization of DNA tumor viruses, such as Simian virus 40 (SV40), is valuable for examining the involvement of key cellular targets in cancer initiation and progression. SV40 is a member of the virus family known as *Polyomaviridae* and was originally discovered in the *Rhesus macaque* monkey (Ahuja et al., 2005). Upon injection into various experimental organisms, SV40 was shown to induce a variety of different tumor types including lymphoma and osteosarcoma (Ahuja et al., 2005). In addition, SV40 induces transformation in cell culture through target of various cellular proteins, making it a powerful resource in cancer cell biology research. The SV40 genome encodes three nonstructural proteins, the large T antigen, a 17K T and the small t antigen (Ahuja et al., 2005). Transformation of rodent cells is solely mediated by the large T antigen and requires its binding to both the p53 and Rb tumor suppressors (Cheng et al., 2009). The large T antigen contains a LXCXE motif which is essential for its interaction with the Rb tumor suppressor (Ahuja et al., 2005). Through direct interaction with Rb, the large T antigen is able to sequester Rb and inhibit it from binding to E2F transcription factors. E2F is then free to activate transcription of its targets, including Myc. As stated above, Myc would then be able to activate transcription of SRSF1, leading to the alternative splicing events that generate the BIN1+12A isoform which lacks pro-apoptotic functions

In our research, the expression of BIN1 isoforms was investigated in cells virally transformed with the SV40 T antigen. Since these cells exhibit an aggressive phenotype, it is thought that they will express higher amounts of the BIN1 tumor isoform, BIN1+12A, when compared to the same immortalized cells not expressing T antigen. This could represent an additional mechanism through which the SV40 T antigen is able to induce cellular transformation due to higher oncogenic Myc activity and less apoptosis.

Materials & Methods:

Our research utilized human diploid fibroblasts (HDF) immortalized with telomerase (htert), which were graciously provided by Dr. Kathleen Rundell (Northwestern University). The HDF(tert) line was transfected with a plasmid containing the entire early region of Simian Virus 40 (pPVU-0) to create the HDF(tert)+T cell line. Thus, HDF(tert)+T was used as our transformed experimental cell line. Cells were maintained in Dulbecco's Modified Eagle Media (10% FBS, 1-2% Sodium Bicarbonate, 1% Pen/Strep mix) and grown at 37°C in a 7% CO_2 incubator. When cells were confluent, RNA was extracted from both cell lines using the RNeasy Plus Mini Kit (Qiagen). Three primer pairs for BIN1 (Weschler-Reya et. al, 1997) were synthesized by the PSU Hershey Medical Center Macromolecular Core Facility and used to perform RT-PCR reactions. The primer pairs corresponded to the 5' region (exons 3-7) of BIN1, the midsection (exons 6-11) and the 3' region (exons 11-16). The sequences of these primers are shown in Table 1. Reverse Transcription-PCR (RT-PCR) was performed using Super-Script

One-Step RT-PCR with Platinum *Taq* (Invitrogen). The RT-PCR products were separated on 1.8% agarose gels and imaged. Due to difficulties cutting out resulting bands on the agarose gel, DNA was purified directly from the RT-PCR products using the QIAquick PCR Purification Kit (Qiagen). This DNA was then inserted into the pGEM-T Easy Vector using the pGEM-T Easy Vector System II (Progema) and transformed into JM109 Competent cells (Promega). These cells were plated on Luria Bertani (LB) agar plates containing 50 µg/ml ampicillin, 50 µg of 20 mg/ml X-Gal and 100 µl of 100mM IPTG. White colonies were selected and cultured overnight in LB broth. DNA was extracted from these overnight cultures using the High-Speed Plasmid Mini Kit (IBI Scientific) and 5 µl of DNA was sent for genomic sequencing at Eurofins Laboratories. Sequences were compared to the known BIN1 sequence and analyzed. Additional 3' products were subject to restriction enzyme digestion with EcoRI and analyzed to determine insert length.

Table 1: Sequences of BIN1 primer pairs utilized for RT-PCR. The 5', midsection and 3' primers correspond to exons 3-7, 6-11 and 11-16, respectively.

Primer	Forward	Reverse
5'	AAGGATCTCCGGACCTACCT	CACATTCATCTCCTCAAACACC
Midsection	TGAAGCCAAAATTGCCAAGGC	TGGCTGAGATGGGGACTTG
3'	GGAGAATTCGCGATGCCTGCAAAAGGGAACAAGAGC	GGACTCGAGTCATGGGACCCTCTCAGTGAAGTTC

Results:

Since SRSF1 levels have been shown to directly affect BIN1 isoform formation, the levels of SRSF1 expression in both cell lines was investigated via Western blotting (Figure 1). HDF(tert)+T cells showed slightly higher expression of the splicing factor when compared to HDF(tert) cells. SRSF1 overexpression would be expected to cause an increase in BIN1 + 12A isoform abundance in the HDF(tert)+T cell line when compared to HDF(tert) cells.

To determine whether the expression of the oncoviral protein T antigen affects the BIN1 isoform produced, RNA was extracted from each of the cell lines and RT-PCR of three sections of the gene transcript was performed. The RT-PCR products from the three primer pairs are shown in Figure 2 on an agarose gel. The 5' and midsection primers produced the expected sizes of 409 bp and 476 bp, respectively. However, the 3' primers produced multiple bands of varying sizes, suggesting the presence of multiple isoforms in both HDF and HDF(tert)+T cells.

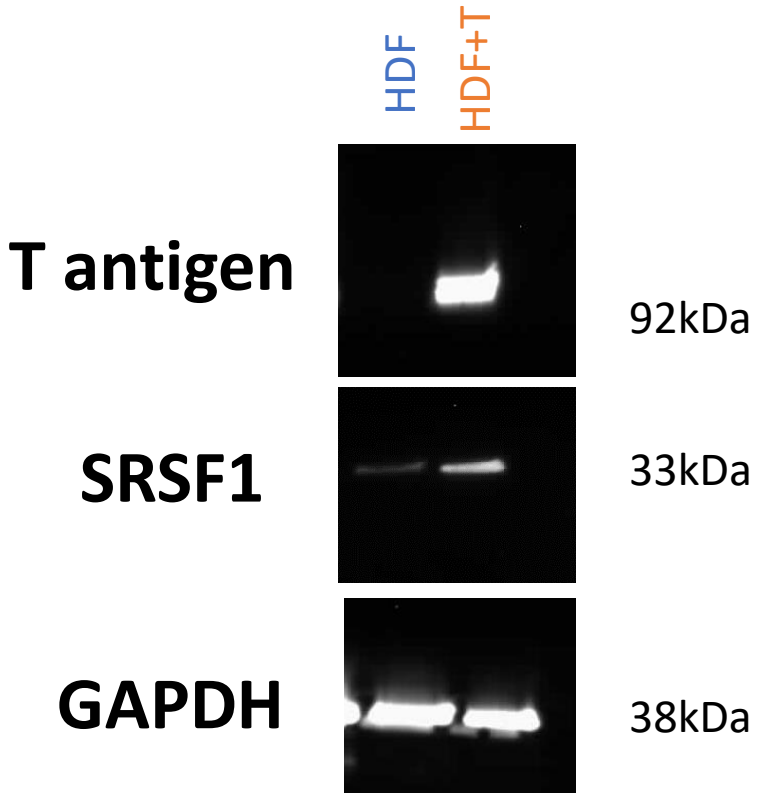

Figure 1: Investigation of SRSF1 levels in HDF(tert) and HDF(tert)+T cells. Western blotting revealed a slight overexpression of SRSF1 (92 kDa) in HDF(tert)+T cells. 50 µg of protein was loaded in each lane. Cell lines were differentiated through the presence of T antigen (33 kDa). GAPDH (38 kDa) was used as a loading control.

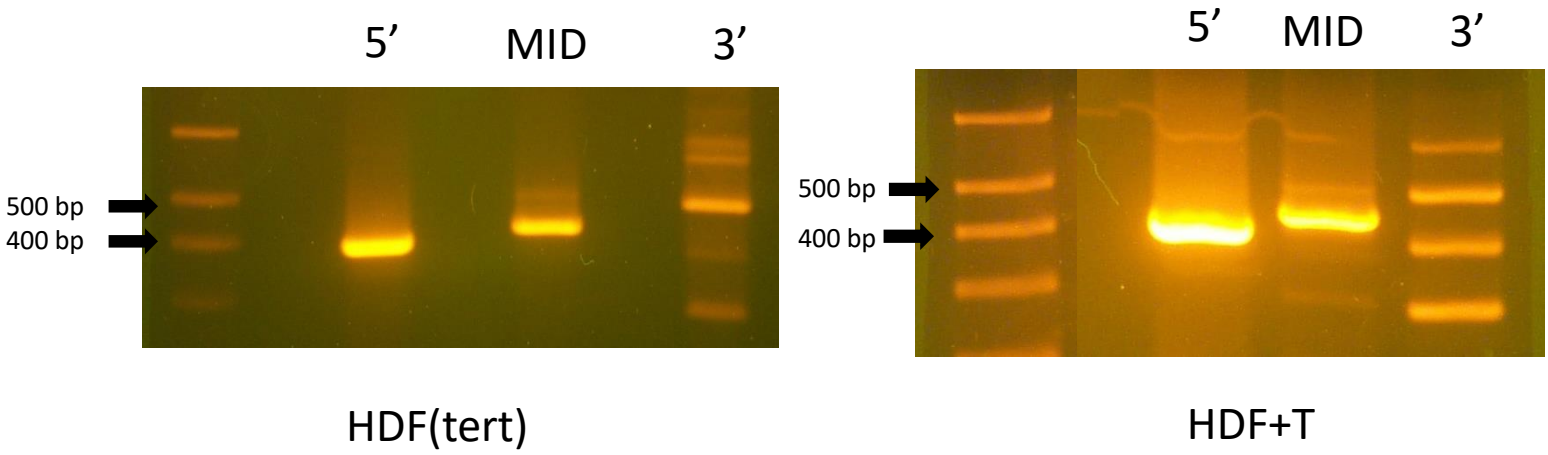

Figure 2: BIN1 RT-PCR products. BIN1 5’, midsection and 3’ RT-PCR products from both HDF(tert) and HDF(tert)+T cells separated on a 1.8% agarose gel. 5’ and midsection products were around the expected sizes of 409 bp and 476 bp, respectively. 3’ primers produced multiple bands, indicating the presence of multiple isoforms in each cell line.

Samples of each RT-PCR product were cloned into the pGEM T easy vector and transformed into competent JM109 cells. White colonies were selected and purified plasmid DNA was sent out for sequencing (Eurofins). Table 2 shows sequencing results from midsection and 3' regions. These sequences revealed the exclusion of exon 10 in HDF(tert)+T midsection products. Additionally, the sequence of one HDF(tert)+T 3' product skipped from exon 11 to 16. This indicates that exon 12A is not present. In addition, it revealed that exons 13, 14 and 15 are absent, suggesting a lack of a MBD in this isoform.

Table 2: Sequences obtained from midsection and 3' products. HDF(tert)+T midsection sequences revealed the absence of exon 10. HDF(tert)+T 3' products revealed the absence of exons 13, 14 and 15, indicating the lack of a MBD.

Cell Line	Midsection	3'
HDF(tert)+T	GGGGGCGAGTCGCATGCTCCGGCCGCCATGGCGGC CGCGGGAATTCGATTTGAAGCCAAAATTGCCAAG**7** GCCGAGGAGGAGCTCATCAAAGCCCAGAAGGTGTT TGAGGAGATGAATGTGGATCTGCAGGAGGAGCTGC CGTCCCTGTGGAACAG**8**CCGCGTAGGTTTCTACGT CAACACGTTCCAGAGCATCGCGGGCCTGGAGGAAA ACTTCCACAAGGAGATGAGCAAG**9**CTCAACCAGAA CCTCAATGATGTGCTGGTCGGCCTGGAGGAGCAAC ACGGGAGCAACACCTTCACGGTCGAGGCCCAGTCC AT**11**TGACACCGCGCCTGCAAAAGGGAACAGGAGC CCTTCGCCTCCAGATGGTTTCCCCTGCCGCCCCCC CTCGAGATCAGACAAAACCACGAGCCCATAGCCCG GCTGGCGGGGCCCCTCCCGGGGGCCTCCGTCGCCT AGTTCCTATTCGTCAGCCAAATTCCTAGTGAAAA	GCTCCGGCCGCCATGGCGGCCGCGGGAATTCGATT CGCGAT**11**GCCTGCAAAAGGGAACAAGAGCCCTTC ACCTCCAGATGGCTCCCCTGCCGCCACCCCCGAGA TCAGAGTCAACCACGAGCCAGAGCCGGCTGGCGGG GCCACGCCCGGGGCCACCCTCCCCAAGTCCCCATC TCAG**16**GATGAAGGCTGGCTCATGGGCGTGAAGGA GAGCGACTGGAACCAGCACAAGGAGCTGGAGAAGT GCCGTGGCGTCTTCCCCGAGAACTTCACTGAAAGG GTCCCATGACTCCAGTCCAATCACTAGTGAATTCG CGGCCGCCTGCAGGTCTACAATANGGGAAAGCTCC CANCGCNTCGGATGTTTATCTTGAGTATTCTATA

Discussion:

RT-PCR confirmed the presence of different BIN1 isoforms in the HDF(tert) and HDF(tert)+T cell lines. Several of these isoforms were able to be successfully sequenced and confirm that we were indeed looking at BIN1. Specifically, the midsection sequence of HDF(tert)+T cells revealed the exclusion of exon 10. However, exon 10 encodes a nuclear localization signal that is reported to be present only in muscle specific BIN1 isoforms (Wechsler-Reya et al., 1997). Thus, since fibroblasts were utilized in this research, exon 10 should be absent. The 3' sequence of the HDF(tert)+T revealed a possible new isoform of BIN1 which does not contain exons 13, 14 or 15 (Figure 3C). Though isoforms lacking exon 13 have been found both ubiquitously in normal tissue and within tumors, there have been no reports of an isoform lacking exons 13, 14 and 15 (Thomas, Chang & Prendergast, 2015). The tumor isoforms which lack exon 13 have not shown growth inhibition of Myc binding capacity since part of the MBD is not present (Lundgaard et al, 2011). Additionally, exogenous expression of BIN1 proteins which completely lack the MBD (ΔMBD) were shown to accumulate to similar levels as full-length proteins,

indicating no protein instability (Elliott et al, 1999). Though overexpression of BIN1ΔMBD proteins does not inhibit Myc activity, MBD-deleted BIN1 has also been shown to inhibit colony forming activities in various human cancer cell lines (Elliott et al, 1999). This indicates that in some instances, BIN1ΔMBD retains its ability to bind Myc and inhibit its activity (Elliot et al, 1999 & Pineda-Lucena et al, 2005). Despite being unable to inhibit transformation by SV40 large T antigen, BIN1ΔMBD can inhibit adenovirus E1A or mutant p53 mediated transformation (Elliot et al, 1999). This suggests possible MBD-independent mechanisms of growth inhibition by BIN1, possibly mediated by a Myc-independent effector domain (MID) encoded in within the BIN1 BAR domain (Lundgaard et al, 2011).

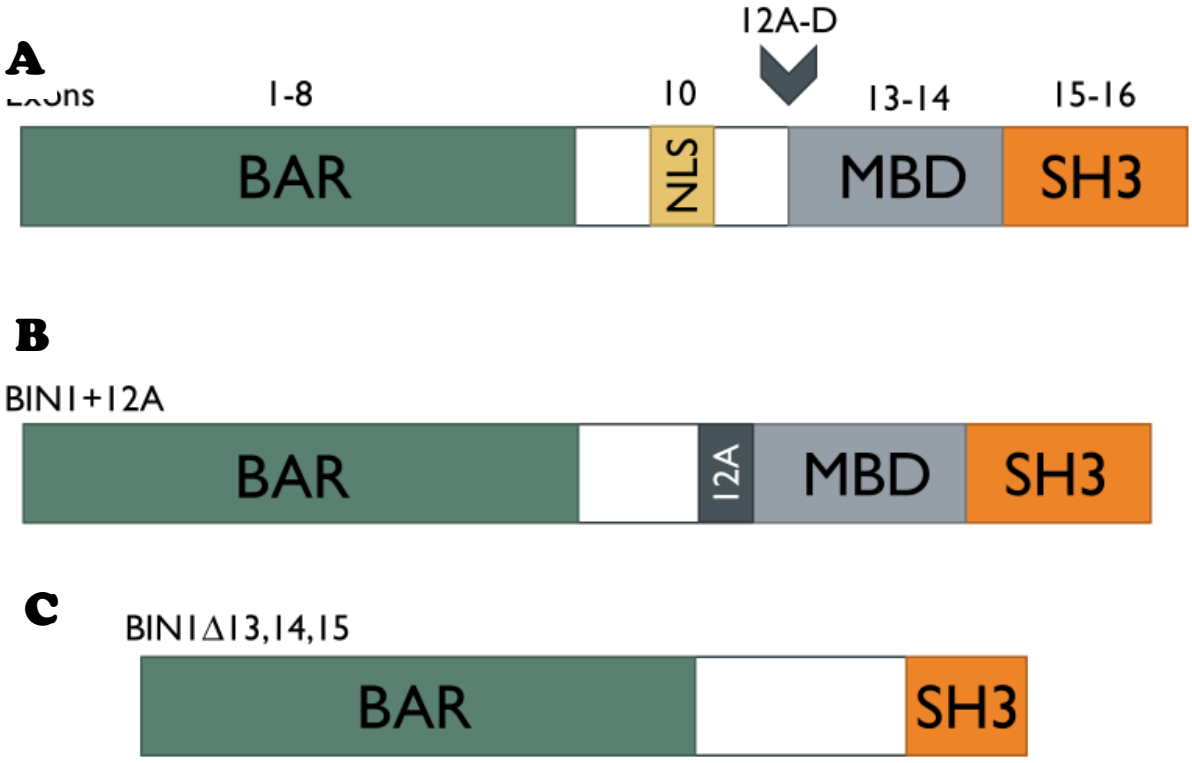

Figure 3: BIN1 exon map. A) Ubiquitous full length BIN1 protein containing an N-terminal BAR domain, a Myc-binding domain (MBD) encoded by exons 13 and 14, and a C-terminal SH3 domain. An arrow indicated where exons 12A-D would be located, if present. B) Known tumor isoform containing exon 12A (BIN1+12A). C) Possible new isoform detected in the HDF(tert)+T cell line which lacks exons 13, 14 and 15. This indicates the absence of a MBD.

Though conformation that the BIN1 isoform lacking exons 13, 14 and 15 (BIN1Δ13,14,15) found in HDF(tert)+T cells forms a function protein is still needed, the transcript does not follow patterns which would suggest nonsense mediated decay. We would expect this isoform to become a multifunctional cytoplasmic protein, since the nuclear localization signal encoded by exon 10 is not present. Additionally, we would assume that BIN1Δ13,14,15 is unable to bind Myc and inhibit its oncogenic abilities, as it lacks an MBD and part of its SH3 binding domain, both of which are known to be integral in the Myc-binding ability of BIN1.

This new BIN1 isoform, BIN1Δ13,14,15 could represent another mechanism by which the SV40 T antigen promotes cellular transformation in our virally transformed model. When the T antigen is present, it is able to bind to the tumor suppressor Rb and inhibit its function, leaving the E2F transcription factor free and active. Active E2F promotes the transcription of Myc, which can function as an oncoprotein and promote cellular transformation. Additionally, E2F is able to activate transcription of BIN1, which in a normal cell is advantageous, since a full length BIN1 protein is able to bind to Myc and inhibit its oncogenic properties. However, the presence of a full length BIN1 protein would not be beneficial for transformed or tumor cells, like our HDF(tert)+T cells. Thus, some tumor cells are known to undergo transformation through the production of a BIN1 isoform containing exon 12A, which is unable to bind to Myc and inhibit its oncogenic properties. An overexpression of the splicing factor SRSF1 in cells transformed with a viral oncoprotein was shown in this research. This SRSF1 overexpression could result in splicing events which produce a unique BIN1 isoform in our model. This research describes the production of a BIN1Δ13,14,15 isoform by HDF(tert)+T cells that is likely unable to bind and inhibit the activity of Myc and therefore promotes cellular transformation.

References:

Anczukow, O., Rosenberg, A., Akerman, M., Das, S., Zhan, L., Karni, R., Muthuswamy, S., Krainer, A. (2012). The Splicing Factor SRSF1 regulates Apoptosis and Proliferation to promote Mammary Epithelial Cell Transformation. *Nature Structural Molecular Biology,* 19(2), 220-228.

Da Luz, F., Brigido, P., Moraes, A., Araujo, R., Silva, M. (2017). Splicing Factors in Breast Cancer: Drivers of the Breast Tumor Fate. *InTech*- Review Article, chapter 11.

Das, S., Anczukow, O., Akerman, M., Krainer, A. (2012). Oncogenic Splicing Factor SRSF1 is a Critical Transcriptional target of Myc. *Cell Replication,* 1(2), 110-117.

Dräger, N., Nachman, E., Winterhoff, M., Bruhmann, S., Shah, P., Katsinelos, T., … Jahn, T. (2017). Bin1 direcly remodels actin dynamic though its BAR domain. *EMBO Reports 18*(11), 2051-2066.

DuHadaway, J., Sakamuro, D., Ewert, D., Prendergast, G. (2001). Bin1 Mediates Apoptosis by c-Myc in Transformed Primary Cells. *Cancer Research,* 61, 3151-3156.

Elliott, K., Sakamuro, D., Basu, A., Du, W., Wunner, W., Staller, P., Gaubatz, S., Zhang, H., Prochownik, E., Eilers, M., Prendergast, G. (1999). Bin1 functionally interacts with Myc and inhibits cell proliferation via multiple mechanisms. *Oncogene,* 18, 3564-3573.

Ge, K., DuHadaway, J., Du, W., Herlyn, M., Rodeck, U., Prendergast, G. (1999). Mechanism for elimination of a tumor suppressor: Aberrant splicing of a brain-specific exon causes loss of function of Bin1 in melanoma. *National Academy of Science,* 96, 9689-9694.

Goncalves, V., Jordan, P. (2014). Posttranscriptional Regulation of Splicing Factor SRSF1 and Its Role in Cancer Cell Biology. *BioMed Research International,* 2015, 1-10.

Karni, R., de Stanchina, E., Lowe, S., Mu, D., Krainer, A. (2007). The gene encoding the splicing factor SF2/ASF is a proto-oncogene. *Nature Structural & Molecular Biology*, 1-7.

Lundgaard, G., Daniels, N., Pyndiah, S., Cassimere, E., Ahmed, K., Rodrigue, A., … Sakamuro, D. (2012). Identification of a novel effector domain of BIN1 for cancer suppression. *Journal of Cell Biochemistry, 112*(10), 2992-3001.

Miller, D., Thomas, S., Islam, A., Muench, D., Sedoris, K. (2012). c-Myc and Cancer Metaolism. *Clinical Cancer Research,* 18(20), 5546-5553.

Pineda-Lucena, A., Ho, C. S., Mao, D. Y., Sheng, Y., Laister, R., Muhandiram, R., … Arrowsmith, C. (2005). A Structure-based Model of the c-Myc/Bin1 Protein Interaction Shows Alternative Splicing of Bin1 and c-Myc Phosphorylation are Key Binding Determinants. *Journal of Molecular Biology,* 351, 182-194.

Tajiri, T., Lie, X., Thompson, P., Tanaka, S., Suita, S., Zhao, H., … Hogarty, M. (2003). Expression of MYCN-interacting Isoform of the Tumor Suppressor BIN1 Is Reduced in Neuroblastomas with Unfavorable Biological Features. *Clinical Cancer Research, 9,* 3345-3355.

Tazi, J., Bakkour, N., Stamm, S. (2008). Alternative Splicing and Disease. *Biochimica er Biophysica Acta,* 1792, 14-26.

Sakamuro, D., Elliott, K., Wechsler-Reya, R., & Prendergast, G. C. (1996). BIN1 is a novel MYC-interacting protein with features of a tumour suppressor. *Nature, 14,* 69-77.

Wechsler-Reya, R., Sakamuro, D., Zhang, J., Duhadaway, J., Prendergast, G. (1997). Structural Analysis of the Human BIN1 Gene. *Journal of Biological Chemistry,* 272, 31453-31458.