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Review Article

Functional Interactions in Transcription and Splicing of Ewing's Sarcoma

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Ewing's sarcoma (EWS) protein is a member of the TET (TLS/EWS/TAF15) family of RNA and DNA-binding proteins with unknown cellular role. EWS protein is encoded by the *EWS* oncogene on chromosome 22q12, a target of chromosomal translocations in Ewing's sarcoma tumors. The exact mechanism of EWS participation in gene expression and pathogenesis of the resulting cancers is not defined. The binding partners of native EWS and EWS fusion proteins (EFPs) are described schematically in a model, an attempt to link the transcription with the splicing. The experimental data about the partnerships of EWS and EFPs are summarized, which may lead to better understanding of their function.

1. Introduction

Transcription and splicing seems to be connected by proteins with roles in both processes, coordinating them, such as TET proteins, since their NTDs mediate interactions with RNA polymerase II (Pol II), while their CTD binds to splicing factors. Thus TET proteins may recruit splicing factors to the Pol II, which coordinates pre-mRNA processing events [1].

The participation of native Ewing's sarcoma protein (EWS) and its oncogenic fusion proteins (EFPs), as well as their reported binding partners in transcription and splicing, could be described schematically by a model to link the transcription with the splicing in ESFTs. Some EWS and EWS/FLI1 interacting partners (including Pol II subunits) are implicated in transcriptome and spliceosome and are directly involved in mRNA synthesis or splicing.

1.1. Ewing's Sarcoma. Ewing's sarcoma, first described by James Ewing in 1921, is still a cryptic malignancy. Ewing's sarcoma family tumors (ESFTs) afflict children and young adults, encompassing Ewing's Sarcoma of bone, extraosseous (soft-tissue) Ewing's sarcoma, primitive neuroectodermal Tumor (PNET), and Askin's tumor. ESFTs have high propensity to metastasize in bone, bone marrow, and lung. ESFTs are aggressive round cell tumors of putative stem cell origin, for which prognostic biomarkers and novel treatments are needed [2, 3]. ESFTs are chemotherapy-sensitive cancers, and even patients with metastatic disease commonly achieve remission. Diagnosis of Ewing tumor is based on pathologic and molecular findings [4]. The EFPs are promoter-specific transcriptional activators, due to EWS-activation domain (EAD) and a DNA-binding domain (DBD) from the fusion partner. About 85% of Ewing tumours carry the most frequent *EWSR1/FLI1* fusion gene that is critically important for maintaining the tumor phenotype of the disease. The *EWS/FLI1* may be fully transforming only in a mutated cell that possesses additional genetic alterations, supported by the experiments with limb transgenic mice and cell culture. The mutations occur after the t(11; 22) translocation occurs (Figure 1), but it is also possible that *EWS/FLI1* transforms a cell that carries a silent and critical mutation in another gene [5]. *EWS*-deficient mice demonstrated that EWS is required for the completion of B cell development and meiosis [6]. *EWS* KO mice displayed aging-like phenotypes, and both tumorigenesis and senescence are intimately tied to stem cells. The loss of endogenous *EWS* results in dramatic changes in the dynamics of hematopoietic stem progenitor cells and a progressive and severe postnatal atrophy of $EWS^{-/-}$ mice hematopoietic organs. $EWS^{-/-}$ cells display impaired DNA repair capacity and show a diminished capacity to sustain

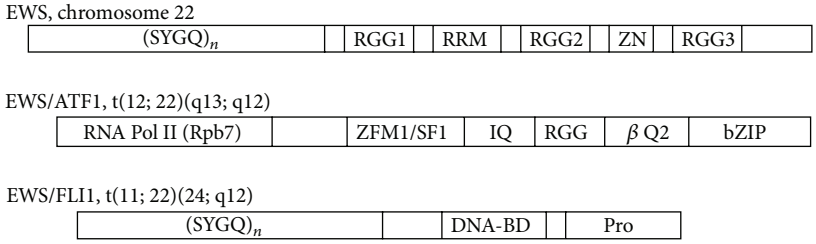

Figure 1: Schematic representation of the functional regions of EWS (656 AA), EWS/ATF1 (537 AA), and EWS/FLI1 (476 AA). The translocation t(11; 22)(q24; q12) encodes the fusion protein EWS/FLI, composed of the NTD of EWS (chromosome 22) fused in-frame to the CTD of FLI (chromosome 11). The translocation t(12; 22)(q13; q12) encodes the fusion protein EWS/ATF1, a fusion in-frame of the NTD of EWS (chromosome 22) to the CTD of ATF1 (chromosome 12). EWS contains an N-terminal transcriptional activation domain (EAD, SYQG rich region; G/P/T rich, 31 tandem repeats) and a C-terminal RNA-binding domain (RBD). The N-terminus of EAD (residues 1–82) binds to RNA Pol II (subunit Rpb7) and to ZFM1/SF1. The RBD contains an RNA-recognition motif (RRM, consisting of about 100 conserved residues), RGG boxes (RGG1, RGG2, and RGG3 of 5, 3, and 12 motifs), and a putative zinc finger (ZN). The EWS/ATF1 is composed of the EAD (residues 1–325, comprising also the IQ domain) fused to the C-terminal region of ATF1 (residues 66–271). The bZIP domain of ATF1 mediates dimerization and DNA binding, Q2 is a glutamine-rich constitutive activation domain, and β is required for activity (contains a critical motif DLSSD). EWS-FLI1 is composed of the EAD (residues 1–264) fused to the CTD of Fli1 (residues 241–452), containing its DNA-BD (DNA-binding domain) and Pro (proline-rich activation domain).

survival of lethally irradiated animals in serial transplantations. *EWS* may play a role in DNA repair and/or DNA recombination [7].

1.2. Native EWS. The EWS is a member of the TET family of RNA and DNA-binding proteins. EWS is located in the nucleus, associated with components of the basal transcription, RNA-splicing factors [8] and G-protein coupled receptor signaling, and relocates from cytoplasm to ribosomes upon Pyk2 activation [9].

EWS consists of an N-terminal transcriptional activation domain (TAD, EAD, NTD, aminoacids (AA) 1–264) and a C-terminal RNA-binding domain (RBD, CTD), extensively methylated at arginine residues, and containing a putative zinc-finger domain [10, 11].

The EAD is intrinsically disordered and resembles many chromatin organizing proteins [12, 13]. Multiple Tyr residues are essential for EAD function [12]. All three RGG motifs of EWS participate in self-association required for the EWS nuclear import [14].

TET family members bind RNA and/or ssDNA in a unique way [15]. The SYGQ-rich TAD of TLS (EWS) may bind RNA, PolII, RPB3, TFIID, Nuclear hormone receptors, while the RBD may bind YB-1, NF-kB, TASR1/2, SC-35 in addition to RNA, ssDNA, and dsDNA [16].

1.3. EWS/ETS Fusions. The generation of chromosomal translocations in Ewing's sarcoma could be mediated by a mechanism of illegitimate recombination, occurring before interchromosomal joining [17]. The resulting EFPs are potent transcriptional activators that interact with other proteins required for mRNA biogenesis. EFPs induce tumorigenesis by perturbing the gene expression and participate in signaling cascades required for oncogenesis [18].

Specific chromosomal translocations fuse EWS to ETS family members (FLI, ERG, FEV, ETV1, and ETV4) in Ewing's sarcomas to give different isoforms of EWS/ETS oncogenic fusions, where the EAD is fused in-frame to the DNA-binding domain (DBD) of the corresponding ETS protein (Figure 1). Around 85% of Ewing's tumours carry the most frequent EWSR1/FLI1 fusion. Phosphorylation, acetylation, and glycosylation are posttranslationally modifying mechanisms, affecting EWS/FLI1 activity. Self-association of EWS and EWS/FLI1 and interaction of EWS/FLI1 with EWS and FLI1 were observed *in vivo,* involving the N-terminal and centrally localized amino acids [19]. The EWS/WT1 phosphorylation on Ser and Tyr is affecting the DNA binding and homodimerization [20]. The transactivation by EWS/ATF1 does not require dimerization, while the bZIP domain is necessary for dimerisation and DNA binding [21].

EWS/FLI1 is acting as a strong transcriptional activator and, in contrast to wt FLI1, is a potent transforming agent [22]. EWS/FLI1 induces gene expression changes by causing smaller alterations in multiple stages of the gene regulation and modulating both transcript synthesis and mRNA degradation. EWS/FLI may have DNA-binding-independent function, blocking the normal function of wt EWS expression [18]. The flexible and unfolded conformation allows EFPs to bind many interacting partners. Direct or indirect target genes of EWS/FLI contribute to tumor growth and progression, such as IGFBP3, GSTM4, CDKN1A, TGFBRII, VEGF, CAV1, E2F8, FOXO1, and NFKBIL2 [23, 24]. The "core" regulators, including genes NR0B1, NKX2.2, and GLI1, are essential for oncogenic transformation in Ewing's sarcoma [18]. EWS/FLI1 repressed miRNAs are targets in IGF signalling pathway, a pivotal driver of Ewing's sarcoma oncogenesis [25]. Both EWS/FLI1 and FLI1 act as antiapoptotic agents, targeting the CBP/p300 pathways *in vitro* and *in vivo* [26].

2. Proposed Link between Transcription Complex and Splicing Machinery in ESFTs

2.1. Native EWS in Transcription and Splicing of Normal Cells. EWS contains an IQ domain that is phosphorylated by protein kinase C (PKC) and is interacting with calmodulin

(CaM), a regulatory link between Ca^{2+} signal transduction pathways and RNA processing [27]. The EWS region interacting with ZFM1 (transcriptional repressor identical to splicing factor SF1) maps to 37 AAs within its NTD (Figure 1). The TET proteins are present in distinct TFIID populations, associated with Pol II holoenzyme, and cooperate with ZFM1 in mRNA processing during transcript elongation [28]. The EAD binds to the coactivator CREB-binding protein, which is implicated in chromatin remodeling, and the hypophosphorylated Pol II [29].

Full-length EWS forms multifunctional complexes with Pol II and TFIID that may be physiologically relevant [15]. EWS interacts with Pol II subunits via hsRpb3. The AAs (1–82) of EWS possess full transforming activity and interact with Pol II. TBP dimerization inhibits the DBD and regulates kinetics of TBP-DNA interaction [30]. EAD binds hRpb5 and directly Rpb7 by yeast two-hybrid screening, coimmunoprecipitation, and pull-down assays [15, 31, 32]. Rpb7 and Rpb4 are required for EAD-mediated transactivation in yeast [33]. Overexpression of recombinant hsRPB7 specifically increases the gene activation by EWS-chimeric TFs [31]. The prolonged hsRpb4/7 depletion is cytotoxic, revealing a gene-specific role in human cell viability [34].

Various genotoxic stress inducers (camptothecin (CPT)) inhibit the interaction between EWS (Pol II-associated factor) and YB-1 (spliceosome-associated factor), resulting in cotranscriptional skipping of MDM2 gene exons, encoding the main p53 ubiquitin ligase. Disruption of the EWS, and YB-1 interaction by CPT may alter the local recruitment of the splicing machinery, affecting MDM2 exon splicing [35]. Proposed is a communication between the transcriptional and splicing machineries mediated by Rpb7, EWS and YB-1 proteins [35]. Proposed are TLS modulation mechanisms of YB-1-directed gene expression of MMP-1 gene, involved in tumor invasion and metastasis, where TLS may be loaded onto nascent transcripts at the promoter, integrating transcript processing and translation [16]. MALDI-MS identified more than 70 prespliceosome-associated proteins, including U1 and U2 snRNP proteins, non-snRNP splicing factors, DEAD-box protein p68, RHA, YB-1 and TLS [36].

Recombinant TAF15 and U1C directly interact *in vitro* (required is NTD of TAF15) [37]. EWS interacts via its RBD with RNase-sensitive protein complexes, consisting mainly of hnRNPs and RNA helicases. HnRNP (M and U), RNA-helicases (p68 and p72), actin, and tubulin interact directly with EWS. EWS and p68 or p72 colocalize in the nucleus of HEK cells [38]. EWS depletion results in alternative splicing changes of genes ABL1, CHEK2, MAP4K2 involved in DNA repair and genotoxic stress signaling [39]. EWS interacts with both the CREB-binding protein (CBP) [26, 29] transcriptional coactivator and also the transcriptional repressor SF1 [28], positively and negatively regulating transcription [1]. RGG domain of EWS is important for G-quadruplex-specific binding, suggesting modulation by specific structures of ncRNAs [40]. It is necessary for *cis*-repression of transcription induced by NTD of the Glu-rich domain, blocking its interaction with hsRPB7. EWS might function as a transcriptional regulator upon its binding with structure-specific RNAs [41].

2.2. EWS/FLI1 in Transcription and Splicing of ESFT Cells. EWS/FLI1 down-regulates gene expression by both transcriptional and posttranscriptional mechanisms, modulating transcript synthesis and degradation, and inducing large gene expression changes by causing smaller alterations in multiple stages of gene regulation [42]. EWS/FLI1 transcriptional complex includes PolII, CREB1, RNA helicase A (RHA) [43] and also BARD1, C-JUN, SAP1a, CBP/p300 as interaction partners [44].

EFPs may disturb gene expression by mimicking or interfering with CTD-Pol II normal function within the transcription initiation complex because EAD shares homology with CTD of Rpb1 [20].

EFPs may contribute to malignant transformation through disruption of RNA splicing, mediated by their binding proteins, such as YB-1 [45] and TASR proteins [46]. Due to interaction with several RNA-processing proteins, including snRNP, U1C, and SF1 [8, 44], EWS/FLI1 activity was linked to RNA transcription and splicing [45]. The U1C expression modulates the EWS/FLI1 transactivation *in vitro* and *in vivo* via interaction with its NTD [8]. RHA protein, a nuclear DNA/RNA helicase, regulating transcription and splicing, was found in a complex with EWS/FLI1 in ESFT cell lines, binding to EWS/FLI1 target gene promoters and enhancing its function as transcriptional cofactor [43]. The complex formation between CBP/p300 and PolII requires RHA as modulator of transcription, inducing local changes in the chromatin structure [43, 47]. EWS/FLI1 specifically binds the RHA fragment AAs (630–1020), and the interruption of this interaction induces apoptosis *in vivo* and *in vitro* in ESFT cells [43].

2.3. The Common Role of EWS and EWS/FLI in Transcription and Splicing of ESFT Cells. EWS is a bridge between basal transcriptional and splicing machinery of the gene expression [8, 15, 28, 45], acting from the transcription initiation through to the delivery of the mature mRNA to the cytoplasm [16]. EWS/FLI may have DNA-binding-independent dominant negative function, blocking the normal function of wild-type EWS expression [18]. EWS is absent in RHA complex from HEK293 cell, while RHA possibly binds to wild-type EWS in ESFT [43]. RHA may function differently in complexes with EWS versus EWS/FLI1, leading to oncogenic transformation in the presence of EWS/FLI1 [43]. EWS/FLI1 and EWS share some protein partners such as BARD1 [48] but uniquely bind others such as YB1 [45].

The EAD of EWS/FLI1 is linked to mRNA splicing via U1C (snRNP) [8], indicating that both EWS/FLI1 and full-length EWS can bind to U1C [49], while their direct interaction remains to be investigated. The functional consequences of heterodimerization between EWS/FLI1 and EWS on RNA splicing also have to be revealed [19, 50].

All experimental data suggest a role of TET proteins in Pol II transcription and splicing, possibly coupling these processes. The participation of EWS and EFPs binding proteins in transcription and splicing could be described schematically by the model shown in Figure 2, as an attempt to link the transcription with the splicing. The partnerships

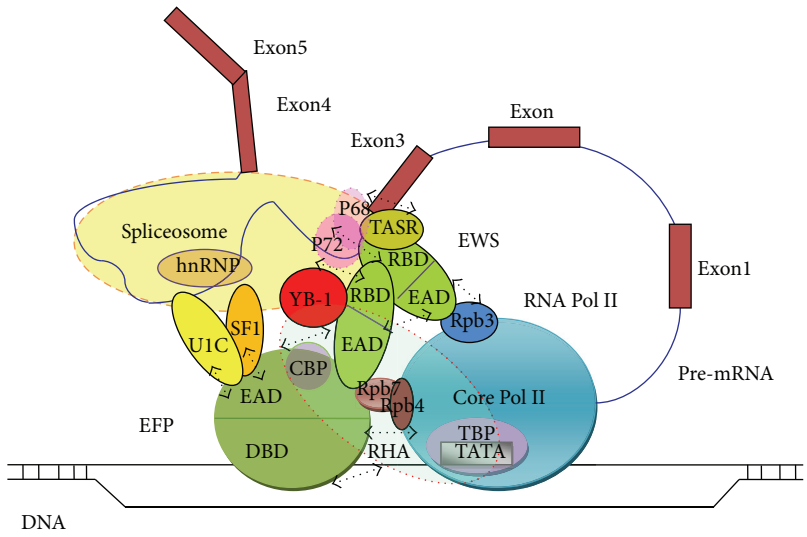

Figure 2: Schematic model of the link between transcription complex and spliceosome, reflecting interaction partners of EWS and EFPs. EWS is a bridge between components of basal transcriptional machinery and splicing apparatus of the general gene expression. EWS protein interacts via its RBD with nuclear ribonucleoproteins (hnRNPs) and RNA helicases. Shown are participants in transcription and splicing: EFP, hnRNPs, RPB7, EWS, YB-1, hnRNPs, RNA helicases, and other binding functional proteins. EWS could be divided into SYGQ-rich TAD and C-terminal RBD. This model shows to which regions of EWS and EFPs the interaction partners preferentially bind. Some interactions are depicted by arrows to better represent the unknown composition and possible flexibility of EWS and EFP containing complexes.

and interactions shown in Figure 2 reflect EWS or EWS/FLI1 binding, either directly or indirectly, while others have to be verified experimentally (the interactions depicted by arrows are unproved, unknown, or flexible). All known transcription activators are not stably associated with TFIID or Pol II.

Evidently, the difference between EWS and EFPs binding to Pol II and TFIID is due to the nature of their CTDs. The EFP complex formation may be the result of structurally independent behavior of EAD and CTD, and their conformational changes. These interactions could be specific to tumor cell and may be undetectable *in vitro* as weak and unstable, allowing or disabling exact complex formations. Such binding element may be the YB-1 protein, connecting the C-terminus of EWS to TFIID and then recruiting them to Pol II, because, in ES cells (expressing endogenous EWS/FLI1), the linkage between YB-1 and Pol II via EWS (or TLS) was found to be defective [45].

The exact mechanisms of action and all interacting partners of EWS and EFPs, their role, and functional relationships in cancer pathways are not well understood. The function and connections of components of the model complexes, including dsDNA, mRNA, Pol II, EWS, EFPs, and other transcription and splicing factors, have to be revealed. The homo- and heterodimerization function of EWS and EFPs in transcription and splicing, and also the direct interaction between EWS/FLI1 and U1 snRNP proteins have to be studied.

3. Conclusions

The proposed hypothetic model is trying to summarize the findings about functioning of EWS and EFPs and helps to understand the transcriptional and splicing mechanisms in humans. An important point is to find the functional consequences of EWS and EWS/FLI1 homo- and heterodimerization on RNA transcription and splicing. The discovery of new interacting partners of EWS and EFPs *in vitro* and *in vivo* as well as the inhibition of interaction, required for oncogenic activity, may help the development of uniquely effective, tumor-specific anticancer agents against Ewing's tumors.

Abbreviations

ESFTs: Ewing sarcoma family of tumors
EFPs: EWS fusion proteins
EAD: EWS activation domain
EWS: Ewing's sarcoma protein
Pol II: RNA polymerase II
NTD: N-terminal domain
TAD: Transcription activation domain
CTD: C-terminal domain
RBD: RNA-binding domain
DBD: DNA-binding domain
CaM: Calmodulin
CPT: Camptothecin
CBP: CREB-binding protein
RHA: RNA helicase A.

References

[1] A. Y. Tan and J. L. Manley, "The TET family of proteins: functions and roles in disease," *Journal of Molecular Cell Biology*, vol. 1, no. 2, pp. 82–92, 2009.

[2] A. Cooper, J. Van Doorninck, L. Ji et al., "Ewing tumors that do not overexpress BMI-1 are a distinct molecular subclass with

variant biology: a report from the children's oncology group," *Clinical Cancer Research*, vol. 17, no. 1, pp. 56–66, 2011.

[3] R. Todorova and A. Atanasov, "Diagnostic strategies and treatment for Ewing's sarcoma," *International Journal of Clinical Medicine*, vol. 3, no. 6, pp. 538–543, 2012.

[4] J. Gonin, F. Larousserie, B. Dousset et al., "An unusual adrenal tumor: Ewing tumor," *Annals of Pathology*, vol. 31, no. 1, pp. 28–31, 2011.

[5] P. P. Lin, Y. Wang, and G. Lozano, "Mesenchymal stem cells and the origin of Ewing's sarcoma," *Sarcoma*, vol. 2011, Article ID 276463, 8 pages, 2011.

[6] H. Li, W. Watford, C. Li et al., "Ewing sarcoma gene EWS is essential for meiosis and B lymphocyte development," *Journal of Clinical Investigation*, vol. 117, no. 5, pp. 1314–1323, 2007.

[7] J. Cho, H. Shen, H. Yu et al., "Ewing sarcoma gene Ews regulates hematopoietic stem cell senescence," *Blood*, vol. 117, no. 4, pp. 1156–1166, 2011.

[8] L. L. Knoop and S. J. Baker, "The splicing factor U1C represses EWS/FLI-mediated transactivation," *Journal of Biological Chemistry*, vol. 275, no. 32, pp. 24865–24871, 2000.

[9] J. S. Felsch, W. S. Lane, and E. G. Peralta, "Tyrosine kinase Pyk2 mediates G-protein-coupled receptor regulation of the Ewing sarcoma RNA-binding protein EWS," *Current Biology*, vol. 9, no. 9, pp. 485–488, 1999.

[10] B. Plougastel, J. Zucman, M. Peter, G. Thomas, and O. Delattre, "Genomic structure of the EWS gene and its relationship to EWSR1, a site of tumor-associated chromosome translocation," *Genomics*, vol. 18, no. 3, pp. 609–615, 1993.

[11] T. Ohno, M. Ouchida, L. Lee, Z. Gatalica, V. N. Rao, and E. S. P. Reddy, "The EWS gene, involved in Ewing family of tumors, malignant melanoma of soft parts and desmoplastic small round cell tumors, codes for an RNA binding protein with novel regulatory domains," *Oncogene*, vol. 9, no. 10, pp. 3087–3097, 1994.

[12] K. P. Ng, G. Potikyan, R. O. V. Savene, C. T. Denny, V. N. Uversky, and K. A. W. Lee, "Multiple aromatic side chains within a disordered structure are critical for transcription and transforming activity of EWS family oncoproteins," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 2, pp. 479–484, 2007.

[13] R. Todorova, "Disorder structural predictions of the native EWS and its oncogenic fusion proteins in rapport with the function," *Advances in Bioscience and Biotechnology*, vol. 3, no. 1, pp. 25–34, 2012.

[14] D. J. Shaw, R. Morse, A. G. Todd, P. Eggleton, C. L. Lorson, and P. J. Young, "Identification of a self-association domain in the Ewing's sarcoma protein: a novel function for arginine-glycine-glycine rich motifs?" *Journal of Biochemistry*, vol. 147, no. 6, pp. 885–893, 2010.

[15] A. Bertolotti, T. Melot, J. Acker, M. Vigneron, O. Delattre, and L. Tora, "EWS, but not EWS-FLI-1, is associated with both TFIID and RNA polymerase II: interactions between two members of the tet family, EWS and HTAF(II)68, and subunits of TFIID and RNA polymerase II complexes," *Molecular and Cellular Biology*, vol. 18, no. 3, pp. 1489–1497, 1998.

[16] W. J. Law, K. L. Cann, and G. G. Hicks, "TLS, EWS and TAF15: a model for transcriptional integration of gene expression," *Briefings in Functional Genomics and Proteomics*, vol. 5, no. 1, pp. 8–14, 2006.

[17] J. Zucman-Rossi, P. Legoix, J.-M. Victor, B. Lopez, and G. Thomas, "Chromosome translocation based on illegitimate recombination in human tumors," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 20, pp. 11786–11791, 1998.

[18] S. Sankar and S. L. Lessnick, "Promiscuous partnerships in Ewing's sarcoma," *Cancer Genetics*, vol. 204, no. 7, pp. 351–365, 2011.

[19] L. Spahn, C. Siligan, R. Bachmaier, J. A. Schmid, D. N. T. Aryee, and H. Kovar, "Homotypic and heterotypic interactions of EWS, FLI1 and their oncogenic fusion protein," *Oncogene*, vol. 22, no. 44, pp. 6819–6829, 2003.

[20] J. Kim, J. M. Lee, P. E. Branton, and J. Pelletier, "Modification of EWS/WT1 functional properties by phosphorylation," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 25, pp. 14300–14305, 1999.

[21] S. Pan, K. Y. Ming, T. A. Dunn, K. K. C. Li, and K. A. W. Lee, "The EWS/ATF1 fusion protein contains a dispersed activation domain that functions directly," *Oncogene*, vol. 16, no. 12, pp. 1625–1631, 1998.

[22] H. Kovar, "Downstream EWS/FLI1—upstream Ewing's sarcoma," *Genome Medicine*, vol. 2, no. 1, article 8, 2010.

[23] L. Yang, H.-M. Hu, A. Zielinska-Kwiatkowska, and H. A. Chansky, "FOXO1 is a direct target of EWS-Fli1 oncogenic fusion protein in Ewing's sarcoma cells," *Biochemical and Biophysical Research Communications*, vol. 402, no. 1, pp. 129–134, 2010.

[24] T. Ravasi, H. Suzuki, C. V. Cannistraci et al., "An Atlas of combinatorial transcriptional regulation in mouse and man," *Cell*, vol. 140, no. 5, pp. 744–752, 2010.

[25] E. L. McKinsey, J. K. Parrish, A. E. Irwin et al., "A novel oncogenic mechanism in Ewing sarcoma involving IGF pathway targeting by EWS/Fli1-regulated microRNAs," *Oncogene*, vol. 30, no. 49, pp. 4910–4920, 2011.

[26] K. L. Rossow and R. Janknecht, "The Ewing's sarcoma gene product functions as a transcriptional activator," *Cancer Research*, vol. 61, no. 6, pp. 2690–2695, 2001.

[27] J. C. Deloulme, L. Prichard, O. Delattre, and D. R. Storm, "The prooncoprotein EWS binds calmodulin and is phosphorylated by protein kinase C through an IQ domain," *Journal of Biological Chemistry*, vol. 272, no. 43, pp. 27369–27377, 1997.

[28] D. Zhang, A. J. Paley, and G. Childs, "The transcriptional repressor, ZFM1 interacts with and modulates the ability of EWS to activate transcription," *Journal of Biological Chemistry*, vol. 273, no. 29, pp. 18086–18091, 1998.

[29] N. Araya, K. Hirota, Y. Shimamoto et al., "Cooperative interaction of EWS with CREB-binding protein selectively activates hepatocyte nuclear factor 4-mediated transcription," *Journal of Biological Chemistry*, vol. 278, no. 7, pp. 5427–5432, 2003.

[30] R. A. Coleman, A. K. P. Taggart, L. R. Benjamin, and B. F. Pugh, "Dimerization of the TATA binding protein," *Journal of Biological Chemistry*, vol. 270, no. 23, pp. 13842–13849, 1995.

[31] R. Petermann, B. M. Mossier, D. N. T. Aryee, V. Khazak, E. A. Golemis, and H. Kovar, "Oncogenic EWS-Fli1 interacts with hsRPB7, a subunit of human RNA polymerase II," *Oncogene*, vol. 17, no. 5, pp. 603–610, 1998.

[32] R. Todorova, "In vitro interaction between the N-terminus of the Ewing's sarcoma protein and the subunit of RNA polymerase II hsRPB7," *Molecular Biology Reports*, vol. 36, no. 6, pp. 1269–1274, 2009.

[33] H. Zhou and K. A. W. Lee, "An hsRPB4/7-dependent yeast assay for trans-activation by the EWS oncogene," *Oncogene*, vol. 20, no. 12, pp. 1519–1524, 2001.

[34] Y. Zhao, K. K. C. Li, K. P. Ng, C. H. Ng, and K. A. W. Lee, "The RNA Pol II sub-complex hsRpb4/7 is required for viability of multiple human cell lines," *Protein Cell*, vol. 3, no. 11, pp. 846–854, 2012.

[35] M. Dutertre, G. Sanchez, M.-C. De Cian et al., "Cotranscriptional exon skipping in the genotoxic stress response," *Nature Structural and Molecular Biology*, vol. 17, no. 11, pp. 1358–1366, 2010.

[36] K. Hartmuth, H. Urlaub, H. P. Vornlocher et al., "Protein composition of human prespliceosomes isolated by a tobramycin affinity-selection method," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 26, pp. 16719–16724, 2002.

[37] M. Leichter, M. Marko, V. Ganou, M. Patrinou-Georgoula, L. Tora, and A. Guialis, "A fraction of the transcription factor TAF15 participates in interactions with a subset of the spliceosomal U1 snRNP complex," *Biochimica et Biophysica Acta*, vol. 1814, no. 12, pp. 1812–1824, 2011.

[38] S. Pahlich, L. Quero, B. Roschitzki, R. P. Leemann-Zakaryan, and H. Gehring, "Analysis of Ewing Sarcoma (EWS)-binding proteins: interaction with hnRNP M, U, and RNA-helicases p68/72 within protein-RNA complexes," *Journal of Proteome Research*, vol. 8, no. 10, pp. 4455–4465, 2009.

[39] M. P. Paronetto, B. Miñana, and J. Valcárcel, "The Ewing sarcoma protein regulates DNA damage-induced alternative splicing," *Molecular Cell*, vol. 43, no. 3, pp. 353–368, 2011.

[40] T. Oyoshi and R. Kurokawa, "Structure of noncoding RNA is a determinant of function of RNA binding proteins in transcriptional regulation," *Cell & Bioscience*, vol. 2, no. 1, article 1, 2012.

[41] X. Wang, S. Arai, X. Song et al., "Induced ncRNAs allosterically modify RNA-binding proteins in cis to inhibit transcription," *Nature*, vol. 454, no. 7200, pp. 126–130, 2008.

[42] K. A. France, J. L. Anderson, A. Park, and C. T. Denny, "Oncogenic fusion protein EWS/FLI1 down-regulates gene expression by both transcriptional and posttranscriptional mechanisms," *Journal of Biological Chemistry*, vol. 286, no. 26, pp. 22750–22757, 2011.

[43] J. A. Toretsky, V. Erkizan, A. Levenson et al., "Oncoprotein EWS-FLI1 activity is enhanced by RNA helicase A," *Cancer Research*, vol. 66, no. 11, pp. 5574–5581, 2006.

[44] H. V. Erkizan, V. N. Uversky, and J. A. Toretsky, "Oncogenic partnerships: EWS-FLI1 protein interactions initiate key pathways of Ewing's sarcoma," *Clinical Cancer Research*, vol. 16, no. 16, pp. 4077–4083, 2010.

[45] H. A. Chansky, M. Hu, D. D. Hickstein, and L. Yang, "Oncogenic TLS/ERG and EWS/Fli-1 fusion proteins inhibit RNA splicing mediated by YB-1 protein," *Cancer Research*, vol. 61, no. 9, pp. 3586–3590, 2001.

[46] L. Yang, H. A. Chansky, and D. D. Hickstein, "EWS·Fli-1 fusion protein interacts with hyperphosphorylated RNA polymerase II and interferes with serine-arginine protein-mediated RNA splicing," *Journal of Biological Chemistry*, vol. 275, no. 48, pp. 37612–37618, 2000.

[47] T. Nakajima, C. Uchida, S. F. Anderson et al., "RNA helicase A mediates association of CBP with RNA polymerase II," *Cell*, vol. 90, no. 6, pp. 1107–1112, 1997.

[48] L. Spahn, R. Petermann, C. Siligan, J. A. Schmid, D. N. T. Aryee, and H. Kovar, "Interaction of the EWS NH2 terminus with BARD1 links the Ewing's sarcoma gene to a common tumor suppressor pathway," *Cancer Research*, vol. 62, no. 16, pp. 4583–4587, 2002.

[49] J. A. Toretsky, "Novel Therapeutic Opportunities for Sarcomas Based on Disordered Chromosomal Translocation Fusion Proteins," American Society of Clinical Oncology, 1092-9118/10/1-10, 2010.

[50] D. Herrero-Martin, A. Fourtouna, S. Niedan, L. T. Riedmann, R. Schwentner, and D. N. T. Aryee, "Factors affecting EWS-FLI1 activity in Ewing's sarcoma," *Sarcoma*, vol. 2011, Article ID 352580, 11 pages, 2011.