

Review Article

EWS-FLI1 Regulates Genotoxic Stress Response in Ewing Sarcoma

Ghosal, Gargi and Jason T. Yustein*

Department of Pediatrics, Baylor College of Medicine, USA

*Corresponding author

Jason T. Yustein, Faris D. Virani Ewing Sarcoma Center at the Texas Children's Cancer Center, Baylor College of Medicine, 1102 Bates Street, Suite 1025.20, Houston, TX 77030, USA, Tel: 832-824-4601; Fax: 832-825-4846; E-mail: yustein@bcm.edu

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Abstract

Chromosomal translocation of the *EWSR1* gene with the members of *ETS* gene family of transcription factors results in the generation of a chimeric transcription factor that underlies the hallmark signature of Ewing sarcoma family of tumors (EFTs). The most predominant translocation and in-frame gene fusion *EWS-FLI1*, occurs between the N-terminal trans-activating domain of EWS and the C-terminal DNA binding domain of FLI1 in Ewing Sarcoma. EWS-FLI1 has been extensively characterized as a transcriptional regulator. However, additional roles of EWS-FLI1 in DNA damage response (DDR), cell-cycle checkpoint control and alternative splicing is only being uncovered now. This review article will discuss the functions of EWS and EWS-FLI1 in genotoxic stress and its potential implications in Ewing Sarcoma oncogenesis and targeted therapy.

ABBREVIATIONS

EFT: Ewing Sarcoma Family Of Tumor; ES: Ewing Sarcoma; DDR: DNA Damage Response

INTRODUCTION

Ewing family of tumors (EFTs), comprising of Ewing sarcoma (ES), Extraosseous Ewing tumor and Peripheral Primitive Neuro ectodermal tumor are a group of aggressive malignant tumor of the bone and soft tissue tumors. Ewing sarcoma is the second most common primary bone cancer that affects children and adolescents. It occurs in the long bones commonly in the tibia, femur, pelvis, humerus, ribs and clavicle or in the soft tissues [1,2]. Pain and swelling in the affected site is frequently the first symptom. While a majority (~ 70%) of Ewing Sarcoma patients with localized disease achieve long-lasting (> 5 years) remissions [2], disease recurrence is still a significant issue. In addition, about 25% of patients present with metastatic disease at diagnosis, most commonly to lungs, bone or the bone marrow and these patients do very poorly, with long term survival rates of less than 30% [2]. Thus, there is still a significant need for understanding the biology of this disease in order to identify new avenues for therapeutic interventions.

The common cytogenetic characteristic of EFTs is the chromosomal translocation of *EWSR1*, at 22q12, with one of the five members from the *ETS* (E26 transformation-specific) gene family of transcription factors namely; *FLI1*, *ERG*, *ETV1*, *ETV5*, *FEV1* [3-5]. *EWSR1* encodes for EWS protein which is a member of the TET (also known as FET) family (TLS/FUS, EWS, and TAF15) of proteins. The domain organization of TET proteins comprises

of a trans-activation domain at the N-terminal and three-RGG box, a RRM-motif, a zinc-finger motif and RNA-binding domain at the C-terminal (Figure 1). TET family of proteins have been shown to interact with transcription factor TFIID, RNA polymerase II [6,7] and components of the RNA splicing machinery [8,9] and have been shown to play key roles in various cellular processes, including gene expression, cell signaling and RNA processing [1]. The ETS family of proteins are well characterized winged helix-loop-helix transcription factors with a highly conserved 85-amino acid ETS domain that mediates site-specific DNA binding and facilitates protein-protein interaction [10]. The ETS proteins function either as transcriptional activator or repressor and have been shown to function in a wide variety of functions that include cell cycle control, cellular proliferation and differentiation and tumorigenesis [8,11,12]. Thus, in normal cells, the members of both TET and ETS family of transcription factors function in cellular processes required for cellular growth, proliferation and differentiation.

However, in 85% of Ewing sarcomas, the chromosomal translocation and in-frame gene fusion between *EWS* and *FLI1* t(11;22)(q24;q12), results in the generation of an aberrant chimeric transcription factor *EWS-FLI1* that harbors the N-terminal trans-activation domain of EWS and the C-terminal ETS DNA binding domain of FLI1 (Figure 1). In rest of the ES cases, translocation is observed between *EWS* and *ERG* t(21;22)(q22;q12) and in rare cases with *ETV1*, *ETV4* and *FEV* ETS family proteins, or similar fusions of the *EWS*-related gene *FUS* (*FUS-ERG* or *FUS-FEV*) [13-16]. Studies from several groups have shown that *EWS/FLI1* and the other fusion proteins are fundamental in Ewing's

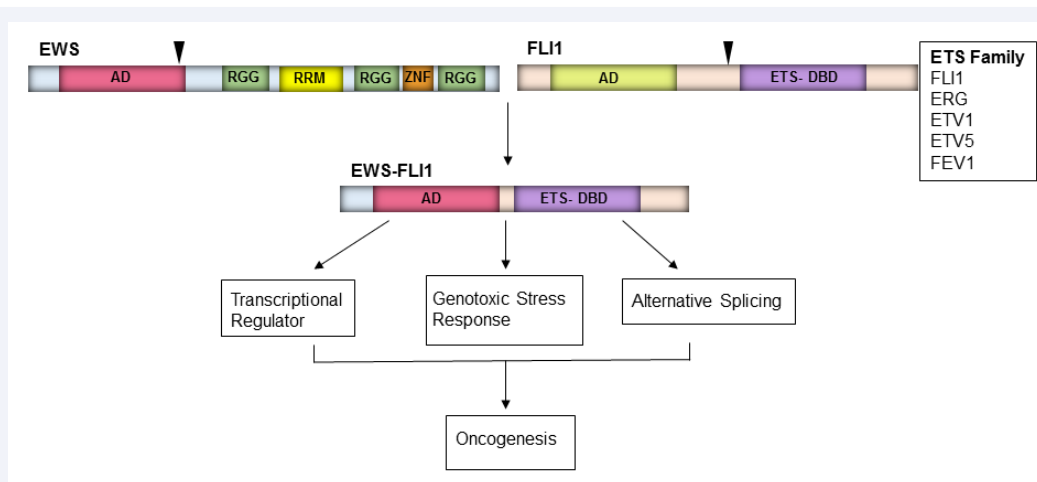


Figure 1 Schematic representation of domain organization and functions of EWS-FLI1 fusion protein in Ewing Sarcoma. The chimeric transcription factor has key molecular functions involved in regulation of direct target genes as well as response to DNA damage and alternative splicing of transcripts that can contribute to oncogenesis. AD, Activation domain; RGG, arginine-glycine-glycine-rich region; RRM, RNA recognition motif; ZNF, zinc finger domain; ETS-DBD; ETS DNA binding domain. Arrows indicate region of chromosome translocation and in frame-fusion.

tumor oncogenesis. Collectively, Genome-wide RNAi screens, Gene expression analysis and ChIP-ChIP studies performed on Ewing sarcoma cell lines and tumor samples show that EWS-FLI1 regulated proteins that range from transcription factors to signaling molecules, cell cycle regulators, DNA damage response and repair, proteins regulating angiogenesis and metabolism- all key players of cellular processes. Dysregulation of these genes by EWS-FLI1 and their biological functions significantly contribute towards driving oncogenesis in Ewing sarcoma [1,8,17].

Though the transcriptional targets of EWS-FLI1 have been investigated in-depth, yet dysregulation of these target genes induced or repressed by EWS-FLI1 does not fully explain the disease phenotype [17]. Since the EWS-FLI1 fusion always harbors only the trans-activating domain of EWS but not the RNA binding region of EWS, it was thought that the RNA processing functions of EWS may not play a role in disease pathogenesis of Ewing sarcoma. However, recent studies imply key roles of EWS and EWS-FLI1 in genotoxic stress and alternative splicing and suggest that the functions of EWS-FLI1 go beyond transcriptional regulation of EWS-FLI1 targets which can further explain sarcoma pathogenesis, mechanisms underlying therapy resistance and identify new targets for therapy. The role EWS-FLI1 in genotoxic stress and its implications will be the focus of this review.

DNA Damage Response

The genome surveillance machinery in the cells, termed DNA Damage Response (DDR), also known as genotoxic stress response, is a network of intricately connected and tightly regulated cellular pathways that include cell cycle checkpoint control, DNA repair pathways, transcription control, alternative splicing and the apoptotic pathway. DNA damage if left unrepaired, result in chromosome rearrangements (chromosome fusions, deletion and mutations) and genome instability. The gross chromosomal aberrations lead to either uncontrolled cellular growth, and cancer development, or cell death. Together, the DDR pathway sense, signals and repairs DNA lesions, thereby ensuring the

faithful maintenance and transmission of the genome from one generation to the next [18,19].

Briefly, DDR involves sensors MRE11/RAD50/NBS1 (MRN complex) that sense the DNA lesions and signals it to the downstream effectors, DDR kinases ATM/ATR, which in turn signal the transducers, CHK1/CHK2 kinases via a cascade of phosphorylation-ubiquitylation signaling events. The activation of CHK1/CHK2 kinases signal the arrest of cell-cycle and transcription machinery and activation of DNA repair pathways and alternative splicing machinery. Depending on the type of lesion, DNA is repaired either by base-excision repair, mismatch repair, nucleotide-excision repair, homologous recombination (HR) and non-homologous end joining (NHEJ) repair pathways. Following DNA repair, active transcription is resumed and cell-cycle is activated, while if the DNA repair pathway fails to fix the damage the apoptotic machinery is activated leading to cell death. DDR proteins and pathways are tightly regulated by protein-protein interactions, post-translational modifications such as phosphorylation, acetylation, ubiquitylation, sumoylation and alternative splicing events [19,20].

EWS protein in genotoxic stress response

The first evidence suggesting a physiological role of EWS in DDR and meiosis comes from the phenotype of *Ews*^{-/-} mice [21]. *Ews*^{-/-} mice show defective pre-B cell development, spermatogenesis and XY asynapsis with increased postnatal lethality, while *Ews*^{-/-} MEFs are hypersensitive to ionizing radiation (IR), and display premature cellular senescence. The phenotypes observed in *Ews*^{-/-} mice are similar to those of *Abl*^{-/-} and *Atm*^{-/-} mice the key players of the DDR and cellular senescence pathways [22-25]. Two separate genome-wide shRNA screens identified EWS as a key protein required for cellular resistance to camptothecin (CPT) and IR treatment [26,27]. Collectively, these observations indicate a role of EWS in meiosis, DDR, recombination mediated repair of DNA break and ageing. However, the exact molecular function of EWS in DDR still remains unclear.

A step closer to understanding the function of EWS in DDR comes from the findings that depletion of EWS results in alternative splicing changes in *CHEK2*, *ABL1* and *MAP4K2*, key regulators of DDR and MAPK signaling [28]. Interestingly, it was observed that cells upon exposure to UV-irradiation display similar alternative splicing changes as observed upon EWS depletion, while knockdown of EWS also sensitized cells to UV irradiation. Moreover, UV treatment results in the transport of EWS to the nucleoli and a corresponding decrease in EWS association with its target sites [28]. Together, these findings suggested that EWS is required for cellular resistance to UV damage. Thus, upon DNA damage CHK2 is activated by phosphorylation and signals the DNA repair machinery by phosphorylating CDC25C, which in turn inhibits the activation of CDKs and arrest cycle progression until the damage is repaired [29,30]. Following DNA repair EWS is removed from the splice sites of CHK2 leading to the reduction of CHK2 protein levels (Figure 2) and subsequent progression of cell cycle. Thus, EWS functions in regulating genotoxic stress response at least in part by regulating the expression of different isoforms of its target genes.

EWS-FLI1 protein in genotoxic stress response

As discussed above, studies clearly demonstrate physiological roles of EWS in meiosis, HR mediated DNA repair and cellular senescence [21]. However, it is not known how these functions of EWS are altered in Ewing sarcoma with the constitutive expression of the potent oncogenic transcription factor EWS-FLI1.

EWS-FLI1 regulates the cell-cycle machinery

Studies show that EWS-FLI1 depleted cells arrest at G0/G1 growth phase [31]. Expression of G1Cyclins- cyclin D and -E

was markedly decreased, while the expression of p21 and p27, G1-S-transition cyclin-dependent kinase inhibitors (CKIs) was dramatically increased both at the mRNA and protein levels upon EWS-FLI1 silencing in Ewing sarcoma cells. Analysis of Ewing sarcoma tumor samples also showed a high level of expression of cyclin D1 mRNA, while p21 and p27 were not detected in the samples. In a subsequent study the authors demonstrated that p21^{WAF1/CIP1} gene is a direct target of EWS-FLI1. Reporter gene assays showed that p21^{WAF1/CIP1} promoter is negatively regulated by EWS-FLI1. The study also showed that EWS-FLI1 interacts with p300 co-transactivator and suppresses the histone acetyltransferase (HAT) activity, thereby altering p21 expression and regulation of the G1-S cell cycle transition. Since, the proper ratio of CDK-cyclin complex to CKIs is critical for G1 progression, an imbalance between the G1cyclin-CDK complex and p21 and/or p27 observed in Ewing sarcoma cell lines and tumor samples clearly indicate that expression of EWS-FLI1 regulates cell-cycle transitions (Figure 2) that results in uncontrolled proliferation and transformation [31].

Functional genomic analysis of gene expression studies performed in Ewing sarcoma cell lines showed an up-regulation of genes involved in cell cycle control (G1-S and G2-M transition), DNA replication, and DNA repair (ATR-BRCA pathway) pathways [32]. Quantitative RT-PCR analysis of *SKP2*, *CDK2*, *MCM10* and *CDC6* showed that these genes were down-regulated upon depletion of EWS-FLI1. It was also noted that these genes were down-regulated in the absence of NROB1, a direct transcriptional target of EWS/FLI1. NROB1 is highly expressed in Ewing's tumors and is a key player of EWS-FLI1 mediated oncogenesis. Since, the levels of EWS-FLI1 remains unaffected in NROB1 depletion, it suggests that the effect of EWS/FLI1 on the expression of these cell-cycle regulators in Ewing sarcoma cells is mainly mediated

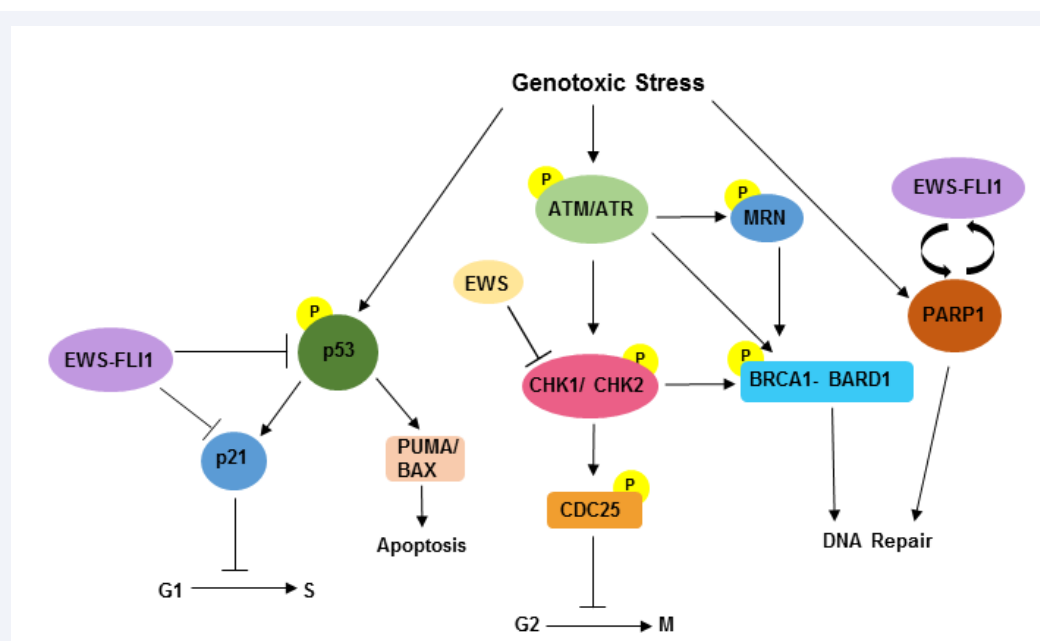


Figure 2 Dysregulation of the genotoxic stress response pathways by EWS-FLI1 contributes to oncogenesis in Ewing Sarcoma. EWS-FLI1 directly suppresses the expression of p21, deregulates the transcriptional activity of p53, regulates the expression of PARP1 in a positive feedback loop mechanism and down regulates the expression of BRCA1 and ATM in Ewing Sarcoma. EWS drives the expression of different CHK2 isoforms by alternative splicing.

through NR0B1 [32]. However, a direct regulation of these cell cycle genes by EWS-FLI1 has not been analyzed and thus cannot be ruled out. Nevertheless, collectively these findings indicate that cell cycle machinery is regulated by EWS-FLI1 and can be selectively targeted for Ewing sarcoma therapy.

EWS-FLI1 and TP53

TP53, plays pivotal roles in preventing cell transformation in the presence of oncogenic or genotoxic stress [33]. *TP53* gene encodes p53 a transcription factor that drives the expression of downstream target genes that activate cell cycle checkpoint pathway, DNA repair, senescence and/or apoptosis in response to DNA damage [33]. DNA damage is a hallmark feature of cancer and thus tumor development and progression frequently requires the abrogation of p53 functions. *TP53* is the most frequently altered gene in cancer. Interestingly, mutations in *TP53* are infrequently found only in 10% of EFTs, nevertheless the p53 pathway is abrogated in EFTs [34,35]. Studies indicate that EWS-FLI1 promotes deacetylation of p53 to inhibit its transcriptional function and protein stability via the recruitment of histone deacetylase 1 (HDAC1). The N-terminal region of EWS-FLI1 is shown to associate with both p53 and histone deacetylase 1 (HDAC1) and overexpression of HDAC1 also significantly inhibits p53 transcriptional activity [36]. Thus, treatment with a pharmacologic inhibitor of HDAC, trichostatin A (TSA) promotes the interaction between p53 and p300, thereby promoting p53-acetylation (p53 lys-382) and subsequent recruitment of modified p53 to promoter regions of its target genes *p21* and *PUMA*, consequently inducing apoptosis. [36]. These results not only provide new insight into the oncogenesis of EFTs by EWS-FLI1 via the inhibition of p53 function (Figure 2), but also lead to the observation that Ewing sarcoma cells can be sensitized by HDAC inhibitors, which are being investigated in clinical trials [37,38].

EWS-FLI1 and DNA repair

Recent findings are only now uncovering the functions of EWS-FLI1 in DNA repair. Initial observations demonstrated the interaction of EWS and EWS-FLI1 via their common N-terminus with the C-terminus of *BARD1*, a putative tumor suppressor [39,40]. *BARD1* and *BRCA1* form a heterodimer via their N-terminal RING finger domains. The *BARD1*-*BRCA1* interaction is essential for the stability of *BRCA1*, a key regulator of HR DNA repair pathway [41]. *EWS*^{-/-} mice phenotype indicate a function of EWS in meiosis and recombination repair of double-stranded breaks (DSBs). However, the molecular function of EWS in meiosis and HR still remains elusive. Caveolin-1 (*Cav-1*), the major component of caveolae, regulates intracellular signaling pathways involved in oncogenic transformation, tumorigenesis and resistance to chemotherapy. *Cav-1* is a target of EWS-FLI1 and drives metastasis in Ewing sarcoma with the production and activation of matrix metalloproteinase [42,43]. *Cav-1* expression also provides resistance to doxorubicin and cisplatin induced apoptosis by the activation phosphorylation of PKC- α in ESFTs [44]. Studies show that *Cav-1* is also regulated by the DDR protein *MDC1*, modulates the activities of HR and NHEJ repair pathways and participate in DNA damage independent mitogen signaling [45,46]. Thus, the interaction of EWS and EWS-FLI1 with *BARD1*-*BRCA1* and the transcriptional regulation of *Cav-1* provides a link

between the EWS-FLI1 and the genome surveillance complex.

EYA3, a DNA repair protein and transcriptional cofactor, is highly expressed in Ewing sarcoma tumor samples and cell lines compared with mesenchymal stem cells. It was found that EWS/FLI1 upregulates *EYA3* by repressing miR-708, a miRNA that targets the 3'UTR of *EYA3*, rather than by binding the *EYA3* promoter directly. The high levels of *EYA3* significantly correlate with low levels of miR-708 in Ewing sarcoma samples. *EYA* proteins are important for cell survival during development and are involved in DNA repair [47]. Thus loss of *EYA3* decreases survival of Ewing sarcoma cells. Most importantly, knockdown of *EYA3* in Ewing sarcoma cells leads to sensitization to DNA-damaging chemotherapeutic drugs used in the treatment of Ewing sarcoma. *EYA3* knockdown cells repair DNA damage less effectively than their control counterparts [48]. These observations identify *EYA3* as a probable mediator and possible biomarker of chemotherapy resistance in Ewing sarcoma. Further studies are required to elucidate the molecular mechanism by which *EYA3* protein mediates resistance to therapy and probable ways to override this resistance.

In 1990, Prasad and colleagues observed that treating Ewing sarcoma cell lines with chemical inhibitors of poly (ADP-ribose) polymerase (PARP), an enzyme involved in DNA break repair and transcriptional regulation, led to an increase in cell death. They also found that the Ewing sarcoma cell lines tested exhibited an increase in PARP1 mRNA transcript with a corresponding increase in PARP1 protein levels and PARP1 enzyme activity [49]. However, it was not until 2012 when a large scale systemic pharmacogenomics profiling analysis revealed that Ewing sarcoma cells harboring the *EWS-FLI1* gene translocation showed a marked sensitivity to PARP inhibitors [50]. In a parallel study, Brenner and colleagues showed that EWS-FLI1 protein associates with PARP1 and demonstrated that Ewing sarcoma cell lines, primary tumor xenografts, and tumor metastases were all highly sensitive to PARP1 inhibition [51]. Furthermore, a combinatorial treatment of PARP1 inhibitor with temozolamide (TMZ), second-line chemotherapeutic agent used in the Ewing sarcoma treatment regimen resulted in complete responses of all treated tumors in a EWS-FLI1-driven mouse xenograft model of EFT. Subsequent functional studies revealed that EWS-FLI1 fusion gene acts in a positive feedback loop to maintain the expression of PARP1 (Figure 2) and oncogene-dependent sensitivity to PARP-1 inhibition [51]. Together, these findings offered a strong preclinical rationale to target the EWS-FLI1/PARP1 intersection as a therapeutic strategy for EFTs. High levels of PARP1 expression are correlated with increased sensitivity to PARP inhibitors that is consistent with a trapping mechanism by which the inhibitor acts as a poison to stabilize a PARP-DNA complex [52-54]. Subsequently, Stewart and colleagues analyzed the cytotoxic activity and *in vivo* efficacy of three different PARP inhibitors (*BMN-673*, *Olaparib*, *Veliparib* currently in clinical trials) in combination with *Irinotecan* (*IRN*) and *TMZ* [55]. The rationale behind the combination therapy lies with the finding that analysis of the Cancer Cell Line Encyclopedia revealed that Ewing sarcoma cell lines show very high levels of *Schlafen-11* (*SLFN11*), a putative DNA/RNA helicase [56]. Previous studies have demonstrated that *SLFN11* expression is positively correlated with increased sensitivity to Topoisomerase I

inhibitors and other DNA-damaging agents, but not protein kinase inhibitors or tubulin poisons [56,57]. It was observed that both TMZ and IRN potentiated PARP inhibitor mediated killing of EWS cells, but higher concentrations of TMZ was required to achieve the same level of potentiation as that achieved with IRN.

Gene expression by qPCR for DNA repair genes confirmed the down regulation of BRCA1, GEN1, and ATM in Ewing sarcoma cell lines [55]. BRCA1 is a key player regulating homologous recombination of double stranded breaks (DSBs) in the DNA generated by IR and drugs such as methyl-methane sulfonate (MMS) and bleomycin. ATM functions as an effector kinase transmitting the damage signal to the DNA repair machinery, while GEN1 is a nuclease that plays key role in recombination repair of DNA breaks (Figure 2). Thus down-regulation of these DNA repair enzymes and effector molecules provide for new attractive targets for therapy to be explored.

EWS-FLI1 drives the expression of the Werner Syndrome protein (WRN) in Ewing Sarcoma cells. WRN is member of RecQ family of helicases and is involved in DNA replication and repair of DNA damage by HR, NHEJ and BER pathway [58]. WRN deficient cells are hypersensitive to CPT. Trabectedin (ET-743, Yondeli) interferes with the activity of EWS-FLI1, reverses the expression of the EWS-FLI1 induced gene signature and blocks the promoter activity and expression of critical EWS-FLI1 downstream targets [59]. Studies with trabectedin also showed downregulation of WRN expression and selectively sensitized Ewing Sarcoma cells to the DNA damaging effects of SN38 (active metabolite of irinotecan). It was found that trabectedin and SN38 are synergistic and demonstrated an increase in DSBs and an accumulation of cells in S-phase. The synergistic effects were translated into the marked regression of Ewing sarcoma xenografts compared to the respective individual drug treatment [60].

Collectively, these findings clearly indicate a role of EWS-FLI1 in regulation of DNA repair pathways and DDR in Ewing Sarcoma. However, the molecular mechanism underlying this regulation is not known and the DNA repair gene targets of EWS-FLI1 are being identified only now. Further validation of the DNA repair gene targets and how EWS-FLI1 regulate them will help decipher the contribution of EWS-FLI1 regulated DNA damage response and repair genes in oncogenesis of Ewing sarcoma, mechanisms of chemo-resistance and uncover new therapeutic targets.

Therapeutic challenge and future perspective

Today a growing body of evidence shows that the functions of EWS-FLI1 is not restricted to mere transcriptional regulation of its targets, but expands to its regulation of alternative splicing, DNA damage response and repair, epigenetic changes (not covered in this review) and cell cycle checkpoint control. An advantage of a disease driven by an oncogenic transcription factor lies in the potential of targeting the oncogenic factor directly or indirectly by targeting the specific downstream targets. Direct inactivation of EWS-FLI1 activity with small molecule inhibitors such as mithramycin and trabectedin are promising [60,61] while, several transcriptional targets of EWS-FLI1 have been investigated as therapeutic targets for Ewing Sarcoma (reviewed in [1,62,63]). Pre-clinical studies with IGF1R inhibiting antibodies were promising, however early clinical trials with IGF1R inhibiting

antibodies as a monotherapy gave a modest response [64]. Subsequently, combination with temsirolimus or rapamycin, inhibitors of mammalian target of rapamycin (mTOR) that acts downstream of IGF1R signaling showed durable responses in refractory disease [65,66]. Tyrosine kinase inhibitor ABT-869 or imatinib mesylate to target c-KIT and platelet derived growth factor receptor, which are both highly expressed in ES [67,68], did not yield significant clinical responses as a monotherapy in ES patients [69,70]. Combination therapy with either conventional drugs (vincristine, doxorubicin, cisplatin, radiation) or other biologically targeted agents such as the death receptor ligand TRAIL is promising in *in vitro* studies [71,72]. Recently, promising pre-clinical studies showing increased sensitivity of Ewing sarcoma cell lines and xenograft tumor models to PARP inhibitors has led to several ongoing Phase I and II clinical trials of Ewing Sarcoma with PARP inhibitors including BMN-673, Olaparib, Veliparib (ABT-888) alone and in combination with TMZ reviewed in [73-75].

The current concern in the field of cancer chemotherapeutics lies in development of therapeutic resistance. One of the established mechanisms mediating resistance to chemotherapeutic agents is DNA repair. Since, most chemotherapeutic drugs work by damaging the DNA, efficient removal of the drug or the damage caused by the drug potentiates resistance. One of the ways to override this resistance would be to target two or more cellular pathways in a synthetic lethal approach. As discussed above EWS-FLI1 promotes G1-S transition by negatively regulating the expression of p21, by promoting deacetylation of p21 mediated by HDAC1. Thus, p21 can serve as a target for therapy for EFTs with the HDAC inhibitors (Figure 2). Where in, Ewing sarcoma cells have been found to be hypersensitive to treatment with HDAC inhibitors [37]. A combinatorial approach of targeting PARP1 with that of HDAC inhibitors could provide for a targeted synthetic lethal approach with efficient killing of cancer cells wherein treatment with PARP1 inhibitor will block DNA repair and activation of the apoptotic machinery will occur with the rescue of p21 levels with HDAC1 inhibitor.

Gene expression analysis of DNA repair genes revealed that BRCA1, GEN1 and ATM are down regulated in Ewing sarcoma cells [57]. BRAC1, GEN1 and ATM play key roles in repair of DSBs by HR and thus decreased expression would imply that Ewing sarcoma cells have a defective HR pathway (Figure 2). PARP1 functions to repair single-strand DNA breaks (ssDNA) in the genome. If the breaks are left unrepaired, the ssDNA eventually leads to the generation of spontaneous DSBs which is then repaired by HR [18]. With HR pathway being defective as a result of down regulation of BRCA1, GEN1 and ATM, a combinatorial treatment of PARP1 with IR, will ensure increased cell death with a synthetic lethal targeted approach.

CONCLUSION

EWS-FLI1 has key roles in transcriptional regulation, cell cycle checkpoint control, DNA damage response and repair and alternative splicing. However, the precise molecular function of EWS-FLI1 in several of these processes is still elusive. Importantly, not much is known about the post-translational modifications of EWS-FLI1 protein and its regulation which in-turn is a key arm in understanding pathogenesis of Ewing sarcoma. It is also

imperative to understand the function of EWS-FLI1 in comparison to its full-length counterparts, EWS and FLI1 in order to identify unique differences in mechanism of action and molecules they regulate. Insights into these pathways will not only help further our understanding of the biology underlying Ewing Sarcoma, but uncover mechanisms that govern resistance to therapy and help identify strategies and new targets for therapy and overcome drug resistance.

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