www.journalmsr.com

الجمعية السعودية لجراحة العظام
Saudi Orthopedic Association

Journal of Musculoskeletal Surgery and Research

Review Article

Genetic alterations in Ewing's sarcoma: What do we know so far?

Abdulmohsen G. Alhejaily, PhD.[1], Eyass M. Yassin, MSc.[1]

[1]Department of Basic Medical Science, Faculty of Medicine, King Fahad Medical City, Riyadh, Saudi Arabia

***Corresponding author:**
Abdulmohsen G. Alhejaily, Department of Basic Medical Science, Faculty of Medicine, King Fahad Medical City, Riyadh, Saudi Arabia.

aalhejaily@kfmc.med.sa

Received: 17 November 2021
Accepted: 26 January 2022
EPub Ahead of Print: 25 February 2022
Published: 21 May 2022

DOI
10.25259/JMSR_148_2021

Quick Response Code:

ABSTRACT

Ewing's sarcoma (EwS) is the second most common cancer that affects bone in children and adolescents. EwS is an aggressive malignancy with a projected overall survival of 70% for the low risk localized and 30% for the metastatic tumors. EwS is genetically described by its unique translocation that fuses FET family genes with the ETS family genes encoding transcription factors. The most frequent molecular event in over 90% of EwS cases is the translocation between EWSR1–FLI1. Additional events, ingincluding *TP53*, mutations, and *CDKN2A* deletions, have been reported but in low incidences. Hitherto, new research on EwS molecular processes is needed to lead to early diagnosis, disease monitoring, treatment development, improve patients' survival, and quality of life. In this review, we outline the presently available information on the genetics underlying EwS carcinogenesis, investigate the existing understanding of the genetics underpinning EwS, and discuss the future directions for research on EwS.

Keywords: Ewing's sarcoma, EWS-ETS, EWSR1-FLI1, Bone cancer, Chromosomal translocation

INTRODUCTION

Ewing's sarcoma (EwS) (OMIM: 612219) is a highly aggressive cancer that develops in bone and soft tissue and affects children and adolescents at a higher rate than the general population.[1] Studies have shown that genetic alterations are the main cause of this cancer. EwS, in most instances, has a non-specific clinical course.[1] Patients may have localized discomfort, often modest, rising somewhat at night or after a workout, but some individuals might have no pain at all. Without alarming pain, the sole indication may be the accidental development of a solid lump.[2] Blood tests may reveal increased levels of indicators of inflammation such as alkaline phosphatase and erythrocyte sedimentation rate.[3] Individuals affected with localized EwS have a good chance of being cured in about two-thirds of cases.[1] Others with solitary metastases might have a long-term survival rate of around 30%, while those with more widespread malignancy that typically involves the bone marrow have a cure rate of fewer than 20% with the available therapies.[4]

The radiologic examination is often more suggestive, with the characteristic of multiple lytic bone lesions. Computed tomography (CT) and/or magnetic resonance imaging are usually used to assess metastatic disease, disease progression, and response to therapy.[5] However, the histologic and molecular examination of biopsy specimens or surgically removed tumor tissue is used to confirm the diagnosis. The biopsy of malignant tissues is essential for a definite

How to cite this article: Alhejaily AG, Yassin EM. Genetic alterations in Ewing's sarcoma: What do we know so far? J Musculoskelet Surg Res 2022;6:117-20.

diagnosis of the tumor. This may be accomplished through a fine needle or core biopsy from the primary location under the guidance of ultrasound or CT.[6] The biopsy is taken for routine cytogenetic analysis, and under the microscope, sheets of small, rounded cells with a visible nucleus and minimal cytoplasm are usually seen on histologic inspection [Figure 1].[7] Yet, a definitive diagnosis relies mostly on the identification of the distinct molecular signature, which is the chromosomal translocations utilizing either in fluorescent *in situ* hybridization (FISH) or the more rapid quantitative polymerase chain reaction (qPCR).[1] Even though FISH can detect *EWSR1* rearrangements, in some instances, it cannot identify all partners of fusion events in EwS, but qPCR is considered to be a better method for detecting the fusion breakpoint and fine-tuning since they are more sensitive than FISH.[8]

EwS is distinguished by its unique reciprocal translocation known as the t(11;22)(q24;q12), which fuses the *EWSR1* on chr22 with the *FLI1* on chr11, this event is predisposing for the carcinogenesis of this disease [Figure 2].[9] EWS-FLI1 is a chimeric oncogene that is involved in a variety of regulatory processes and is linked to secondary events, which results in the EwS phenotype.[9] In addition to the primary genetic alteration, the accumulation of secondary genetic alterations involving several genes was found to have a more significant impact on the EwS clinical and biological behavior than the fusion gene alone.[10] In this review, we outline the presently available information on the genetics underlying EwS carcinogenesis, investigate the existing understanding of the genetics underpinning EwS, and discuss the future directions for research on EwS.

GENETIC ALTERATIONS IN EWS

Chromosomal translocations

On the molecular level, EwS is marked by the fusion hallmark of the *EWSR1* gene with one of the ETS families

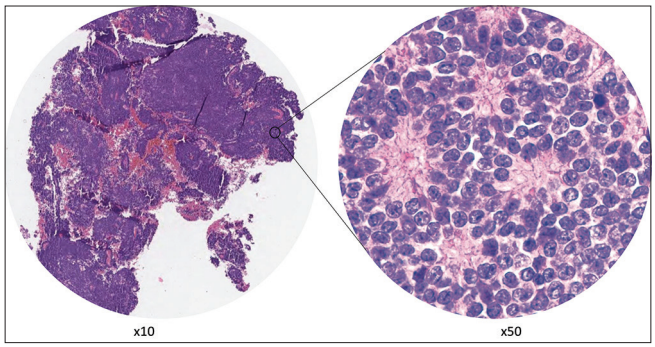

Figure 1: Microscopic (histologic) images of Ewing's sarcoma (EwS) tissue stained with (H&E); showing composed of sheets of small round blue cells that distinguish EwS. The image courtesy of PathologyOutlines.com, Inc.

of transcription factors. The most frequent fusion is the EWSR1-FLI1. This fusion is the result of the chromosomal translocation entails the *FLI1* gene that encodes a transcription factor associated with cell proliferation, growth, and tumorigenesis, and the *EWSR1* gene that encodes a protein involved in several cellular processes such as cell signaling and transcription. Tissues are found to have the EWSR1-FLI1 chromosomal event in over 90% of EwS cases.[11] Additional translocations involving the *EWSR1* gene such as t(21;22) (q22;q12)) result in the formation of the fusion gene EWSR1-ERG, as well as fusions with additional genes such as *ETV1*, *E1AF*, and *FEV* in 5–10% of these cases.[11] Additional chromosomal translocation among EWS-ERG fusion has been well documented as well but with very low incidences.[11]

Based on a study done by Akiko *et al.*, which indicated that EWS-ETS fusion proteins activate telomerase activity in tumors through over-activation of *TERT* gene expression. When the chimeric EWSR1-FLI1 gene is expressed, a protein that blocks the apoptosis pathway is produced, resulting in uncontrolled tumor development, and this protein, in turn, stimulates the transcription of the *TERT* gene, which favorably regulates the expression of telomerase, thus increasing the immortality of tumor cells in EwS.[12] A strong transcriptional activation domain is gained by the EWSR1-FLI1 and the binding domain is provided by FLI in the context of this fusion protein. For EWSR1-FLI to operate as an oncogene, both of these domains must be present to back up the hypothesis that the fusion protein works as a transcription factor.[13]

Interestingly, in EwS cases where EWSR1-FLI1 does not exist, alternative events, mostly translocations, are present that fuse *EWSR1* to another member of the ETS family, such as the *ERG*, *ETV1*, *ETV4*, and *FEV* with very low incidences.[14] To ascertain further potentially EwS causing mutations, Mitchell *et al.*, at the Center for Cancer Research (CCR) in the National Cancer Institute launched a large-scale study protocol on a cohort of (2000 cases) for children and young adults, enrolled in CCR clinical trials to sequence their tumors DNA to ascertain mutations that underlie EwS carcinogenesis. They identified additional three loci at 6p25.1, 20p11.22, and 20p11.23, with low frequency associated with increased EwS risk. This study finding support the inherited genetic to EwS risk and proposes the prominence of the interaction among germline variation and the acquired EWSR1-FLI1 translocations to the etiologic EwS.[15]

Additional genetic alterations

Despite the prominence of EWSR1-FLI1 fusion for the initiation of EwS, this fusion alone found not to be enough to transform human cells *in vitro*, suggesting that the additional secondary alteration or related parallel pathways might be

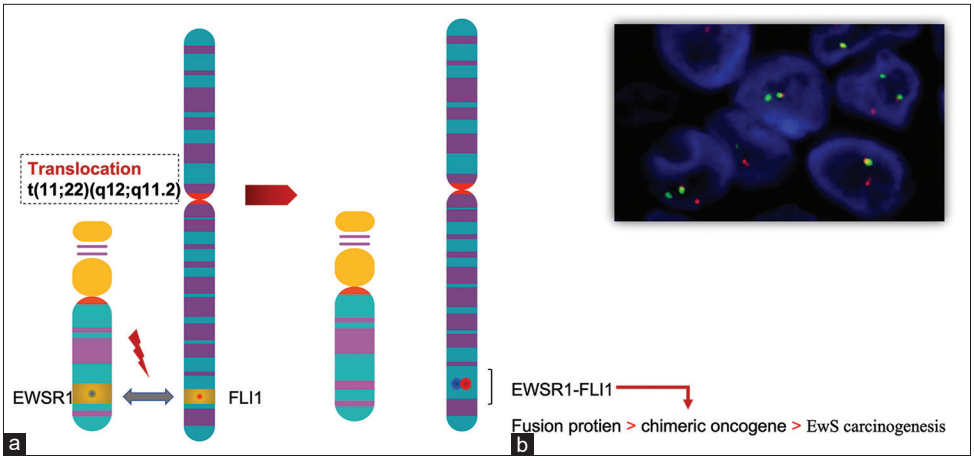

Figure 2: (a) Ewing's sarcoma is distinguished by its unique reciprocal translocation known as the t(11;22)(q12;q11.2), which fuses the *EWSR1* on chr22 with the *FLI1* on chr11. This event predisposes for the carcinogenesis of this disease. This EWSR1/FLI1 fusion is a chimeric oncogene that is involved in a variety of regulatory processes and is linked to the EwS phenotype. (b) EWSR1/FLI1 rearrangement shown by fluorescence *in situ* hybridization (FISH) technique (this part of the figure (b) or FISH image courtesy of PathologyOutlines.com, Inc.).

crucial for EwS pathogenesis a high rate of likely pathogenic mutations, accounting for 13.1% of the population.[16]

EwS has been the subject of various studies, all of which have shown that the tumors have a relatively silent genome, with a scarcity of mutations in pathways that would be susceptible to therapy with innovative targeted treatments. These articles discovered recurrent genomic abnormalities in several genes, including studies that showed EWSR1-FLI1 fusion stimulates tumor cells' production of a protein known as pappalysin-1 (PAPPA), which is responsible for breaking down proteins known as insulin-like growth factor binding proteins (IGFBPs).[17] This breakdown results in the production of the hormone-IGF-1, which, in turn, promotes the proliferation of cancer cells. Through utilizing cell and animal models, researchers were able to effectively show that inactivating PAPPA is a feasible therapeutic option for EwS.[17]

Further mutations in the tumor suppressor genes such as *CDKN2A* and *TP53* have been identified as a secondary genetic alteration in EwS in multiple studies. By utilizing a combination of whole-genome sequencing and targeted sequencing approaches, Hemelin *et al.* found a homozygous deletion affecting *CDKN2A* in 13.8% and 50% and mutations of *TP53* in 6.2% and 71.9% in both EwS' clinical tumors and cell lines, respectively.[18,19] Another study by Brohl *et al.*, the same group examined the sequencing data from the genomes of 175 Ewing patients, discovered a high rate of pathogenic or likely pathogenic mutations in 13.1% of patients.[16] Some of these patients possess genetic mutations in DNA repair genes. However, their analysis in both studies showed that EwS has a very low mutational burden in general (0.15 mutations/Mb).

EWS-FLI1 tends to activate genes involved in cellular differentiation, proliferation, and survival, such as *IGF1*, *NKX2*, *TOPK*, *SOX2*, and *EZH2*. EWS-FLI1 also tends to deactivate genes involved in apoptosis and cell cycle, including the *IGFBP3*, *CDKN1C*, *CDKN1A*, and *TGFB2*.[18,19] Furthermore, because the EWSR1–FLI1 fusion is constitutively active, it is found to work as an activator of the *MYC* gene, which is a proto-oncogene that is essential for embryonic development but that relies on extra external and internal stimuli to become activated its ability to function. Although the *MYC* gene has decreased activity in differentiated cells, its inappropriate activation results in gene amplification, which results in the expression of proteins that are engaged in the control of the cell cycle, differentiation, and genomic instability, which might promote the tumor initiation.[20]

CONCLUSION

EwS is a rare and highly fatal disease that has a poor prognosis when metastatic or recurrent. EwS also serves as a good model for studying carcinogenesis because it has several features that are both helpful and significant. For example, almost all cases (>90%) of EwS have the t(11;22)(q24;q12) chromosomal translocation, which encodes the EWSR1-FLI oncoprotein. However, apart from the t(11;22) translocation, many instances have generally straightforward karyotypes with no other discernible anomalies. It also seems that underlying genetic predisposition to EwS, if it exists, must be a very uncommon occurrence. Much has been revealed about the biology of EwS over the past three decades, including the key role of oncogenic EWSR1-FLI1 fusions that have an extent and importance of the carcinogenesis process in this disease. This information opens the door to targeted therapies, including blocking IGF signaling and, more

recently, interfering with key EWSR1-FLI1 protein-protein interactions conserved pathways in cancer which is likely to suggest additional ways to attack EwS therapeutically.

FUTURE DIRECTION

Genomic analysis of EwS is critical to understanding the genetic and molecular alterations associated with this malignancy. NGS-based genetic analysis has the potential to enhance the prognosis, as it can be used to reach a precise and early diagnosis.

AUTHORS' CONTRIBUTIONS

AGA drafted the manuscript and did the preparation. EMY revised the article and helped in the literature review. All authors have critically reviewed and approved the final draft and are responsible for the manuscript's content and similarity index.

ETHICAL APPROVAL

The authors confirm that this review had been prepared following COPE roles and regulations. Given the nature of the review, the IRB review was not required.

DECLARATION OF PATIENT CONSENT

Patient's consent not required as there are no patients in this study.

FINANCIAL SUPPORT AND SPONSORSHIP

This study did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CONFLICTS OF INTEREST

There are no conflicts of interest.

REFERENCES

1. Burchill SA. Ewing's sarcoma: Diagnostic, prognostic, and therapeutic implications of molecular abnormalities. J Clin Pathol 2003;56:96-102.
2. Henk CB, Grampp S, Wiesbauer P, Zoubek A, Kainberger F, Breitenseher M, *et al*. Ewing sarcoma. Diagnostic imaging. Radiologe 1998;38:509-22.
3. Heare T, Hensley MA, Dell'Orfano S. Bone tumors: Osteosarcoma and Ewing's sarcoma. Curr Opin Pediatr 2009;21:365-72.
4. Yu H, Ge Y, Guo L, Huang L. Potential approaches to the treatment of Ewing's sarcoma. Oncotarget 2017;8:5523-39.
5. Bernstein M, Kovar H, Paulussen M, Randall RL, Schuck A, Teot LA, *et al*. Ewing's sarcoma family of tumors: Current management. Oncologist 2006;11:503-19.
6. Wilkins RM, Pritchard DJ, Burgert EO Jr., Unni KK. Ewing's sarcoma of bone. Experience with 140 patients. Cancer 1986;58:2551-5.
7. Amiel A, Ohali A, Fejgin M, Sardos-Albertini F, Bouaron N, Cohen IJ, *et al*. Molecular cytogenetic parameters in Ewing sarcoma. Cancer Genet Cytogenet 2003;140:107-12.
8. Roessner A, Mittler U, Rose I, Radig K, Grote H. Pathology of Ewing sarcoma. Pathologe 1996;17:6-17.
9. Turc-Carel C, Philip I, Berger MP, Philip T, Lenoir G. Chromosomal translocation (11; 22) in cell lines of Ewing's sarcoma. C R Seances Acad Sci III 1983;296:1101-3.
10. Riggi N, Stamenkovic I. The biology of Ewing sarcoma. Cancer Lett 2007;254:1-10.
11. Delattre O, Zucman J, Plougastel B, Desmaze C, Melot T, Peter M, *et al*. Gene fusion with an ETS DNA-binding domain caused by chromosome translocation in human tumours. Nature 1992;359:162-5.
12. Takahashi A, Higashino F, Aoyagi M, Yoshida K, Itoh M, Kyo S, *et al*. EWS/ETS fusions activate telomerase in Ewing's tumors. Cancer Res 2003;63:8338-44.
13. Erkizan HV, Uversky VN, Toretsky JA. Oncogenic partnerships: EWS-FLI1 protein interactions initiate key pathways of Ewing's sarcoma. Clin Cancer Res 2010;16:4077-83.
14. Grunewald TG, Bernard V, Gilardi-Hebenstreit P, Raynal V, Surdez D, Aynaud MM, *et al*. Chimeric EWSR1-FLI1 regulates the Ewing sarcoma susceptibility gene EGR2 via a GGAA microsatellite. Nat Genet 2015;47:1073-8.
15. Machiela MJ, Grunewald TG, Surdez D, Reynaud S, Mirabeau O, Karlins E, *et al*. Genome-wide association study identifies multiple new loci associated with Ewing sarcoma susceptibility. Nat Commun 2018;9:3184.
16. Brohl AS, Patidar R, Turner CE, Wen X, Song YK, Wei JS, *et al*. Frequent inactivating germline mutations in DNA repair genes in patients with Ewing sarcoma. Genet Med 2017;19:955-8.
17. Kirschner A, Thiede M, Grunewald TG, Rubio RA, Richter GH, Kirchner T, *et al*. Pappalysin-1 T cell receptor transgenic allo-restricted T cells kill Ewing sarcoma *in vitro* and *in vivo*. Oncoimmunology 2017;6:e1273301.
18. Hamelin R, Zucman J, Melot T, Delattre O, Thomas G. p53 mutations in human tumors with chimeric EWS/FLI-1 genes. Int J Cancer 1994;57:336-40.
19. Brohl AS, Solomon DA, Chang W, Wang J, Song Y, Sindiri S, *et al*. The genomic landscape of the Ewing Sarcoma family of tumors reveals recurrent STAG2 mutation. PLoS Genet 2014;10:e1004475.
20. Sollazzo MR, Benassi MS, Magagnoli G, Gamberi G, Molendini L, Ragazzini P, *et al*. Increased c-myc oncogene expression in Ewing's sarcoma: Correlation with Ki67 proliferation index. Tumori 1999;85:167-73.