REVIEW ARTICLE

Pathogenesis of Ewing sarcoma: A review

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Keywords
Chimeric protein, Ewing sarcoma, EWS/E-twenty six oncogenic fusions, EWS/FLI, oncogenesis

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Received 11 May 2015;
Accepted 20 June 2015

doi: 10.15713/ins.jcri.70

Abstract

Ewing sarcoma is a rare sarcoma of bone and soft tissue that uncommonly involve the head and neck. It is the second most common malignant tumor seen in children and young adults. It is most common observed during the second or third decade, with a male predilection. In the head and neck region, it involves skull, clavicle, maxilla, and mandible. The reported incidence of this tumor is only 1-3 cases per million of population per year and skull tumors constitute about 2% of it. The cell of origin is still unclear as there are several literature supporting both the neural crest mesenchymal progenitor/stem cells hypothesis. Ewing sarcoma is believed to be derived from recurrent EWS/E-twenty six (ETS) oncogenic fusions, which result in altered gene expression in the cell and other mechanisms. This altered gene expression is followed by the abnormal regulation in the expression of several genes and non-coding RNAs. In Ewing sarcoma, the translocation of t(11;22)(q24;q12) is considered as the primary mechanism for tumorigenesis. The tumor is characterized by the rearrangement of EWS gene on chromosome 22q12 and the fusion partners from the ETS oncogene family, chimeric transcription factor, which is responsible for the Ewing sarcoma oncogenic program. A hybrid gene is formed by the fusion of EWSR1 in 22q12 with the FLI1 gene in 11q24. EWS/FLI is the common chimeric protein that is expressed in Ewing's sarcoma. The expression of this protein results in the growth arrest and cell death, when they are expressed in primary cell lines. The expression of these proteins in primitive cell or tumor cell causes differentiation defects resulting in oncogenesis. In this review, we have made an attempt to have an insight into the possible mechanism of pathogenesis and cell of origin of Ewing sarcoma.

Introduction

Ewing sarcoma is a rare sarcoma of bone and soft tissue that uncommonly involve the head and neck. It is the second most common malignant tumor seen in children and young adults,[1] commonly seen in the second or third decade, with a male predilection. The principal sites of this tumor include long bones of the extremities, paravertebral region, chest wall and vertebrae or the ribs. In head and neck region, it involves skull, clavicle, maxilla and mandible. The mandible is the more common site than maxilla. In the mandible, the posterior body, the angle and ramus regions are most common. Its presentation involves bone expansion, mobile teeth, and fever. The reported incidence of this tumor is only 1-3 cases per million of population per year. The skull tumors constitute about 2% of tumors.[2] The recurrence of this cancer has a survival rate of 10% with increased risk of chronic health conditions.[3] The cell of origin is still unclear. In general, the tumor exhibits rapid growth. It is usually located in the deeper regions and may measure about 5-10 cm in greatest diameter. Superficially cases do occur, but are rare. In few cases, pain can be observed. It may result in the disturbances of sensory or motor nerves, when they involve spinal cord or peripheral nerves.[4] Ewing sarcoma belongs to the Ewing sarcoma family of tumors, which are considered as morphologically heterogeneous tumors[5] that are characterized by chromosomal translocations involving the EWS gene with a member of the E-twenty six (ETS) family genes[3] which results in the major alterations in the cell gene. The dysregulated signaling of receptor tyrosine kinase involving insulin-like growth factor-1R, PIK3R3, PTEN and PIK3R have a key role in Ewing's sarcoma pathogenesis. Altered pathways of RB and p53 may also be responsible factors for mutation in Ewing sarcoma.[6] However, the cell of origin and the particular mechanism of pathogenesis is still unclear, for this tumor.[7] In this review, we have made an attempt to have an insight into its cell of origin and also the possible mechanism in the pathogenesis of Ewing sarcoma.

History

In 1918, a case was reported by Arthur Purdy Stoui which was associated with the ulnar nerve. The tumor was composed of round cells, which were undifferentiated and arranged in the form of rosettes in a 42-year-old man. After 3 years James Ewing reported a round cell neoplasm was noticed in a girl of about 14 years. They named it as "diffuse endothelioma of bone" and proposed an endothelial derivation hypothesis. In 1975, Angervall and Enzinger described the first Ewing sarcomas arising in soft tissue (extra-skeletal). This tumor bears the name Ewing and is most common called as Ewing's sarcoma. However, during conversation as well as in literatures both the terms are used interchangeably. Both the terms have been used interchangeably in previous literature. Etiology Ewing sarcoma is believed to be derived from recurrent EWS/ETS oncogenic fusions, which results in altered gene expression in the cell and other mechanisms may result in the altered regulation of several genes and non-coding RNAs.[6,8-10] Recently, Ewing sarcomas are also known to exhibit genetic alterations other than EWS/ETS oncogenic fusion 1. The tumor is characterized by the rearrangement of EWS gene on chromosome 22q12 and the fusion partners from the ETS oncogene family. FLI1 on or ERG on chromosome 21q22 (10%) are most frequent involved.[11] This fusion results in chimeric transcription factor which is responsible for Ewing sarcoma oncogenic program.[11]

The cell of origin

The recognition of a more appropriate model system involved in the pathogenesis of this tumor still remains unclear due to the lack of knowledge in identifying the cell of origin. The cellular environmental factors that influence the expression of EWS/FLI are unclear. In many primary cells, the expression of EWS/FLI causes growth arrest or cell death. The differentiation defects are resulted by the increased expression of these proteins, in the primitive cells.[12] The presentation of Ewing's sarcoma as an undifferentiated "small round blue cell tumor" will present an idea about its original cell of origin.[12] In 1921, its endothelial origin was first proposed by James. Later, various authors have suggested different theories regarding its original cell of origin. They consider that cell of origin of this tumor might be hematopoietic,[13] fibroblastic,[14] neural crest,[15] and mesenchymal progenitor/stem cells.[16,17] There is an increase in the number of evidences supporting the theory of mesenchymal progenitor/stem cells origin.[18] The expression of EWS-FLI1 is associated with activation of some genes and repression of other genes demonstrating the complexity of cellular response to this oncogenic transcription factor.[19] Later studies have demonstrated the development of tumor with a small round cell morphology by the transduced cells in an immunocompromised mice following the introduction of EWS/FL1 protein into the bone marrow cells mesenchymal progenitor cells.[17,20] Few studies have also demonstrated the expression of CD99 in Ewing's sarcoma, but the harboring of CD99 in the murine genome is still a controversy. It is observed that the human mesenchymal stem cells (MSCs) will also provide a suitable cellular context for the expression of EWS/FL1. MSCs are also capable of retaining their propogation, even in the presence of the EWS/FL1 protein. These cells exhibit gene expression profiles which are similar to that of Ewing's sarcoma. However, they are distinct from the other tumors of bone and soft tissue[21,22] and these cells failed to develop tumors they were injected into an immunocompromised mice[21] suggesting the necessity of EWS/FLI and may not be sufficient for oncogenic transformation of human MSCs. Recently, it has been demonstrated the possibility of development of Ewing's sarcoma either from a neural crest stem cells or from MSCs [Table 1].[5,6,13-15,17] Several studies supported this theory. The authors have also observed the expression of antigens on the cell surface, which are found to be associated with the neuroectodermal lineage. Their expression was found in Ewing sarcoma.[23-25] Recently, a study on gene expression profile found that, the genes that were seen expressing in neural tissues as well as during the differentiation of neurons were also expressed in Ewing sarcoma, in a large amount. They also observed that there was an upregulation of neural genes with the expression of EWS/FLI.[26] Based on their latter observation, they suggested a different hypothesis. The expression of EWS/FL1 will result in the Ewing's sarcoma neural phenotype, hence does not reflect the cell of origin.[27-29] Due to the lack of knowledge about the definite cell of origin, the analysis of early stage precancerous cells cannot be accomplished. In other sarcomas also, similar approach has been effective in identifying the definite cell of origin as in the case of rhabdomyosarcoma,[30,31] synovial sarcoma,[32] and osteosarcoma.[33]

Pathogenesis

Aurias *et al.* and Turc-Carel in 1983, first described the translocation of t(11;22)(q;24;12) in Ewing sarcoma.[34] This was thought to be the primary mechanism for tumorogenesis. However, the heterogeneous biology found in the tumors of patients with ES suggests the involvement of other molecular mechanisms.[6] Ewing sarcoma was the first sarcoma that was characterized by the translocation at the molecular level. The translocation occurs in t(11;22)(q24;q12). A hybrid gene is formed by the fusion of EWSR1 in 22q12 with the FLI1 gene in 11q24. EWSR1 gene is a member of ten-eleven translocations (TET) and has multiple functions. TET family

Table 1: The various proposed cell of origin

Cell of origin	Proposed by	Year
Endothelial origin	James	1921
Hematopoietic origin	Kadsin and Bensch	1971
Fibroblatic	Dickman *et al.*	1982
Neural crest origin	Cavazzana *et al.*	1988
Mesenchymal progenitor/stem cells	Castillero-trejo *et al.*	2005
Neural derived MSC or from a neural Crest cell	Elizabeth C. Toomey	2010

MSC: Mesenchymal stem cell

proteins are involved in the processing and transportation of RNA, expression of genes and signaling of cells. FL1 belongs to the family of ETS. These are the transcription factors and DNA sequences are targeted by them.[35] The 50th portion of EWSR1 is joined to FLI1 by t(11;22)(q24;q12). Hence, the domain of transcription activation will be replaced by the sequences of EWSR1. There will be variation of breakpoints in two genes. The fusion between EWSR1 exon 7 and FLI1 5 or 6 are most common. The alterations in the translocation will result in the EWSR1 gene with other different ETS genes like ETS-related gene, ETS-variant gene 1 or 4, or fifth Ewing sarcoma variant. Among these fusions, EWSR1 and ERG fusion are most common [Figure 1].[28,36,37] In spite of having genetic diversities, the structure of these alternative fusions are similar to EWS/FLI1. The retrospective study comparing these variants did not reveal any significant differences in their clinical presentation and overall survival and event-free survival.[37] The translocation in the chromosomes between EWSR1 and transcription factors gene will result in the increase of chimeric proteins will increase in their number due to the and are known to involve in tumorigenesis through their function as an aberrant transcription factor.[34]

McKinsey *et al.* stated that precursor (pri-mr) transcription was downregulated by the repression of EWS/FLI1 group. This was consistent with a transcriptional regulatory mechanism.[37] Nakatani *et al*. have analyzed and demonstrated five miRs. They

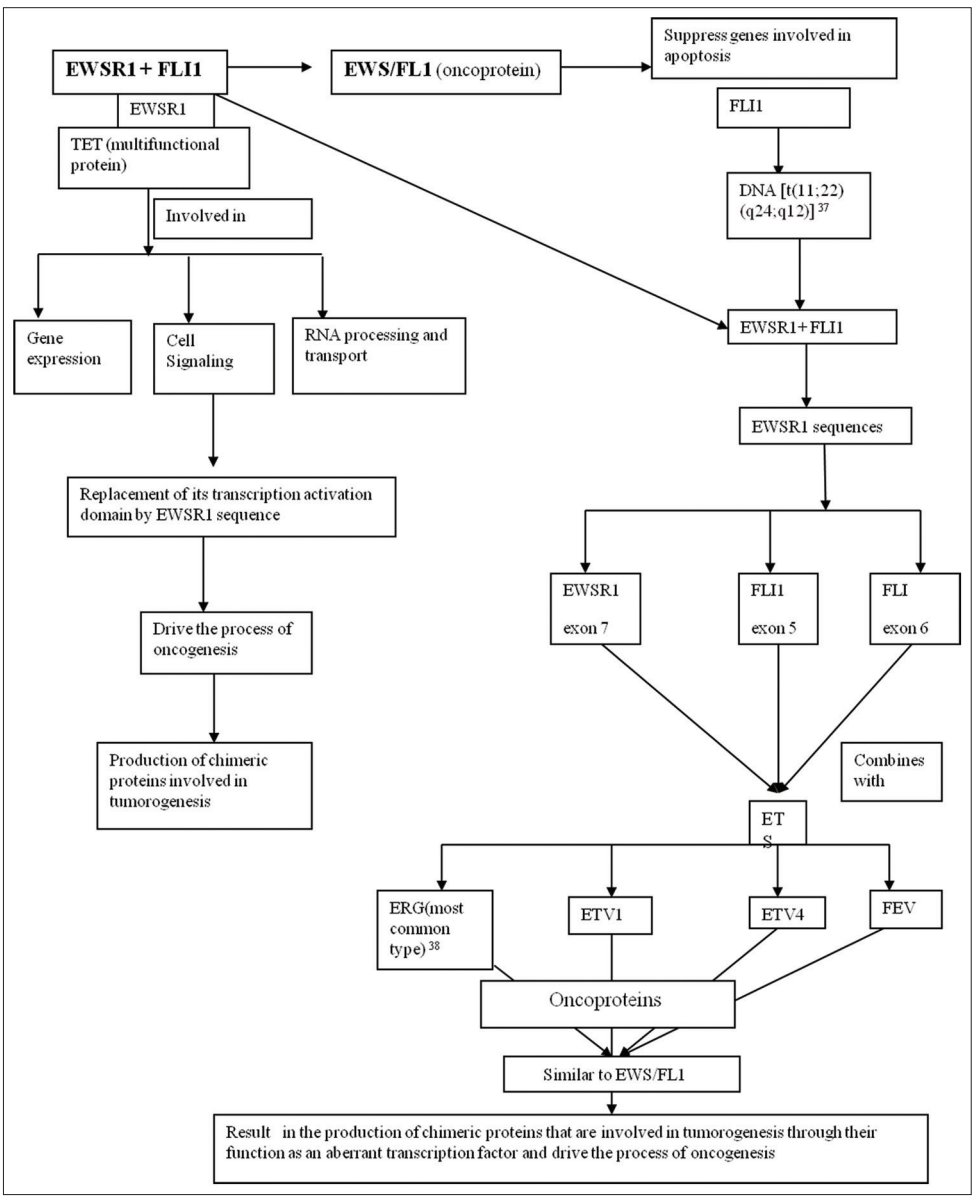

Figure 1: The sequence of molecular events reported during the pathogenesis of Ewing sarcoma

are, 34a, 23a, 92a, 490-3p, and 130b. The significant association of miR with event –free and overall survival was observed.[38] They have also observed the low level of miR-34a which appears as a predictor of early relapse.[39] In other studies, the authors have reported that the Ewing sarcoma cell line's anchorage-independent growth was inhibited by the expression of miR-34a. The expression of miR will be regulated by p53 and its role was consistent with miR-34a levels. The levels were low in Ewin sarcoma cell lines. Inactivation mutations of p53 were also found.[9] The role of EWS in miR processing and whether its haploinsufficiency, affects the miR biogenesis processing function in case of Ewing sarcoma, is yet to be determined. The protein-protein interaction of EWS/Ets-EWS, causing the interference or gain to processing function is also yet to be determined.[9,40]

Conclusion

Ewing sarcoma is a common and complex disease of bone and soft tissues affecting children and young adults. The definite cell of origin is yet to be determined. The cell of origin is known to play a significant role in the pathogenesis the disease by providing a suitable environment for the transcriptional abnormal regulation, which is mediated by EWS/FL1. It also involves the other altered signaling pathways resulting in different genetic diversities makes the research about this study a challenge. The treatment of this disease has still remained a challenge. The better definite knowledge about its cell of origin the potential of cancer stem cell in Ewing sarcoma will facilitate to study this malignancy which helps in the better understanding of the disease. This will allow the researchers to gain knowledge about the various proteins and their contributions in the tumorigenesis of Ewing's sarcoma. Finally, new technologies should be developed to identify the mutations that cooperate with EWS/FL1 including its targets. New advanced studies should be performed for the identification of any alternate mechanisms that can prevent the extensive proliferation and other phenotypes of cancer. The enhanced molecular insight about this disease can facilitate a much better and targeted treatment in treating this disease.

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How to cite this article: Rani PS, Shyamala K, Girish HC, Murgod S. Pathogenesis of Ewing sarcoma: A review. J Adv Clin Res Insights 2015;2:164-168.