The Investigation of EWS-FLI-1 Fusion Gene in the Ewing Family of Tumors

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ABSTRACT There is evidence that 95% of the Ewing family of tumors (EFT) have a EWS-FLI-1 fusion gene. EWS-FLI-1 is a transcription factor with a pivotal function and it is known to bind to a special DNA sequence. Research has demonstrated that the EWS-FLI-1 fusion gene occurrence is related to the EFT, and it has been used to diagnose, treat and serve as a basis for EFT prognosis. We have briefly summarized the progress of the EWS-FLI-1 fusion gene in basic and clinical investigation within the past several years.

KEYWORDS: Ewing family of tumor, fusion gene, EWS-FLI-1.

Overview

Since the report of the Philadephia chromosome (PH chromosome) in 1960, most of the new fusion genes, which have been identified, are found in leukemia and malignant lymphoma. With the development of molecular biology, many specific translocations of chromosomes have been found in solid tumors, especially in soft-tissue sarcomas derived from mesoderm (Table1).[1]

The Ewing family of tumors (EFT), which derive from neuroectoderm, include classic Ewing's sarcoma, Askin tumors of the chest wall, and peripheral primitive neuroectodermal tumors of bone or soft tissues.[2,3] The first case of Ewing family of tumors was described nearly 80 years ago, but now it occurs annually in the USA at a frequency of 2.7 cases per million children under the age of 15 years annually in the USA. Although EFT can occur at any age, the great majority of cases occur at less than 20 years of age. Approximately 95% of EFT have a specific translocation, t (11; 22)(q24; q12), which results in fusion of the EWS and FLI-1gene and production of a new fusion protein EWS-FLI-1. Experiments and clinical cases have shown that EWS-FLI-1 is involved in tumorigenesis and progression of EFT, and it has become used for diagnosis, treatment and a basis of prognosis.[4-7]

Basic investigation of the EWS-FLI-1 fusion gene

The specific t(11;22) (q24;q12) chromosomal translocation occurring in 95% of EFT is caused by rearrangement of the 5' half of the EWS gene on chromosome 22q12 with the 3' half of the FLI-1 gene on

Table 1. Partial list of the chromosome translocations and fusion genes in soft-tissue sarcomas

Tumor type	Translation	Gene fusion	Incidence
ES/PNET	t(11;22)(q24;q12)	EWS-FLI-1	95%
Desmoplastic small round-cell tumor	t(11;22)(p13;q12)	EWS-WT1	95%
Myxoid liposarcoma	t(12;16)(q13;p11)	TLS-CHOP	95%
Extraskeletal myxoid chondrosarcoma	t(9;22)(q22;q12)	EWS-CHN	75%
Malignant melanoma of soft parts	t(12;22)(q13;q12)	EWS-ATF1	NK
Synovial sarcoma	t(X;18)(p11.23;q11)	SYT-SSX1	65%
Synovial sarcoma	t(X;18)(p11.21;q11)	SYT-SSX2	35%
Alveolar RMS	t(2;13)(q35;q14)	PAX3-FKHR	75%
Alveolar RMS	t(1;13)(q36;q14)	PAX7-FKHR	10%
Congenital fibrosarcoma and mesoblastic nephroma	t(12;15)(p13;q25)	ETV6-NTRK3	NK

PNET: primitive neuroectodermal tumor; NK: not known; RMS: rhabdomyosarcoma.

chromosome 11q24.[1] The 5' half of the EWS gene contains a transformation domain which contains an abundance of glutamine and the 3' half of the EWS gene includes a TATA-binding protein (TBP) code typical of a RNA-binding factor. EWS is a functional RNA-binding protein that, along with TLS and a novel TATA- binding protein-associated factor, TAFII68 (also known as RBP56), forms a new family of related proteins. Both TLS and EWS can function as TATA-binding protein-associated factors (TAFIIs).[4] This suggests that EWS may mediate the steps of nascent transcripts with the basal transcription apparatus. However, the EWS portion of EWS-FLI-1 may be redirected to other protein targets because of chromosomal conformation changes induced by the gene fusion.[8] FLI-1 is a member of the ETS (erythroblastosis virus-associated transforming sequences) family of transcription factors which can activate specific target genes by binding to their cognate DNA sequences through their DNA-binding regions. Recent research has shown that FLI-1 has a close relationship to cell immortalization resulting in cancer. Studies also have shown that FLI-1 has a relation to vascularization and growth of neuroectoderm. The 5' half of the FLI-1 gene contains a transformation domain and the 3' half of the FLI-1 gene is a DNA-binding domain (DBD). The ETS family members are defined by the presence of a highly conserved 85-amino acid domain named the ETS domain. The conserved domain is the main part of the DBD in the ETS family. Computer analysis shows that the N-terminal of the ETS domain has a α helical structure and the C-terminal has a conserved structure. The DNA sequence bound by ETS contains a purine-rich sequence centered around a conserved GGAA/T polynucleotide and is not the same in different members of the ETS family.[9] In the EWS-FLI-1 fusion protein, the RNA-binding motif containing the C-terminal half of EWS is replaced by the DBD of the FLI-1 protein. The EWS-FLI-1 is formed by the transformation domain of EWS and DBD of FLI-1. It is believed that the EWS-FLI-1 is a new transformation factor, which can recognize a special domain upstream from the gene and can activate the transformation. In the EWS-FLI-1 fusion protein, both the N-terminal domain of EWS and the DBD of FLI-1 are necessary for the transforming activity. Research has indicated that the activation of transformation by the EWS-FLI-1 is much stronger than that of the FLI-1 in normal cells, and it even can transform cultured NIH3T3 cells.[10] To ensure that the DBD domain in the 3' end of FLI-1 in Ewing's sarcoma can recognize and bind with a special gene sequence, Mao et al.[11] tested the binding activity of 22 different gene sequences and found that ACCGGAAGT was the best.

It has been demonstrated in clinical studies, that various EFTs have different clinical behaviors, progression and therapeutic efficacies. These characteristics have a close relation to the different fusion types that are caused by variations in the

locations of the EWS and FLI-1 genomic breakpoints. Melot et al. [12] reported that at least 12 types of EWS-FLI-1 chimeric transcripts had been observed in clinical investigations, and the fusion of EWS exon 7 to FLI-1 exon 6(type 1) or FLI-1 exon 5 (type 2) accounted for about 60% and 25% of EWS-FLI-1 fusions respectively. The different fusion types have no significant difference in biological activity and the fusion of the N-terminal domain of EWS, encoded by exons 1-7, with DBD of FLI-1, encoded by exon 9, are the minimal components need for activety. The difference in clinical behavior, progression and therapeutic efficacy for EFT is due to the components of EWS and FLI-1. The clinical behavior and therapeutic efficacy of fusion type 1 is found in tumors less malignant. In 1999, Lin et al.[4] used an electrophoretic mobility shift assay(EMSA) to detect the radioactivity of the isotope-labeled DNA, containing the ACCGGAAGT sequence, in 7 different EWS-FLI-1 fusion types of EFT and found that they were almost the same. They also constructed a reporter plasmid pS2 by ligating the ACCGGAAGT sequence into the SacI and KpnI sites of pGL3-Promoter (Promega). To compare the ratio of luciferase activity, the pS2 was transferred using liposomes into 7 different Ewing's sarcoma cells and control cells (Hela and NIH3T3). The results showed that the ratio of luciferase activity in the Ewing's sarcoma cells was much higher than that in control cells ($P = 0.003$) and that the ACCGGAAGT was the best binding sequence of EWS-FLI-1.

Clinical investigation of the EWS–FLI–1 fusion gene

It is obvious that the molecular diagnosis of cancer depends on special molecular markers. Because special molecular markers used for diagnosis are few, the clinical application of molecular diagnosis is limited. Fusion genes of tumors caused by special chromosomal translocations are highly specific and have a potential of becoming a tool for molecular diagnosis, histological typing and judging therapeutic efficacy, reoccurrence and prognosis.[13] Clinical researches are now focusing on how to use the EWS-FLI-1 fusion gene to determine the optimal EFT therapy.

Clinicians have difficulty in diagnosis, treatment and prognosis of EFT, because the histological and immunohistochemical differences are few between EFT and the small blue round cell tumors (SBRCP). We know that 95% of EFT have a specific chromosome translocation, (11; 22) (q24; q12), and that the fusion of EWS and FLI-1 leads to high expression of FLI-1. To evaluate the expression of FLI-1 in 132 SBRCP determined by pathology, Folpe et al. [14] used anti-FLI-1 polyclonal antibody and immunohistochemical technology. They found that the expression of FLI-1 was seen in 29 of 41 (71%) ES/PNET, 7 of 8 (88%) lymphoblastic lymphomas, 1 of a single (100%) desmoplastic round-cell tumor (it has the EWS-FLI-1 gene), 0 of 8 poorly differentiated synovial sarcomas (PDSS), 0 of 32 rhabdomyosarcomas (RMS), 0 of 30 neuroblastomas (NB), 0 of 8 esthesioneuroblastomas (ENB), 0 of 3 Wilms' tumors and 0 of a single mesenchymal chondrosarcoma. Their research demonstrated that immunohistochemistry could distinguish EFT from SBRCP.

At one time antibodies for CD99 were suggested as a specific marker for EFT, but now we know that it is not specific. The expression of CD99 is on the cell membrane but EWS-FLI-1 is in the nucleus. Specificity for a nuclear marker is greater than one on the cell membrane. For example, NB and ENB tumors are positive when tested by FLI-1 antibody but they are almost always negative when tested by CD99 antibody. The expression of FLI-1 is also helpful in distinguishing EFT from other tumors that may be CD99-positive, such as PDSS.

In recent years, autologous bone marrow transplantation has been used to treat EFT. To avoid re-implantation of the tumor cells and EFT re-occurrence, one must make sure that no tumor cells remain in the graft. Traditional technology has been used to detect the existence of the EFT cells, but the sensitivity is not high, around 1% . Using RT-PCR technology, Vicha et al. [7] found the EWS-FLI-1 fusion gene to be negative in 31 bone marrow samples and 5 blood samples, and 7 different fusion types were found in 7 autologous bone marrow transplantations. This

research suggests that RT-PCR is more sensitive than histology in detecting the existence of EFT cells, and a more important assay in conducting autologous bone marrow transplantations.

CONCLUSIONS

The EWS-FLI-1 fusion gene is found in 95% of EFT and this fusion gene is fundamental for the occurrence and development of EFT. EWS-FLI-1 is a transcription factor that has a potent activity by binding with a special DNA sequence which leads to a high expression of itself and the down-stream genes. The EWS-FLI-1 has already been used in the diagnosis, treatment and prognosis of EFT, and is becoming a target for gene therapy. Gene fusion is very common in cancer. The important goal now is how to utilize the knowledge about the EWS-FLI-1 fusion gene in relation to EFT as well as to other tumors.

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