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Molecular diagnosis of pediatric tumors

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ABSTRACT

Pediatric tumors are challenging in the context of best diagnosis, treatment, and prognosis. For tumors which have a genetic association or a cancer predisposition syndrome, the prognosis depends on accurate diagnosis. The application of molecular genetics to pediatric tumors has resulted in better diagnostic and prognostic factors for patient management. Molecular diagnostic techniques, such as reverse transcription polymerase chain reaction and fluorescence *in situ* hybridization (FISH), have become important tests for childhood tumors. Targeted therapies are aimed at specific translocations which are detected by FISH. Molecular techniques help in monitoring of minimal residual disease in childhood tumors.

Key words: Fluoresence in situ hybridization, Paediatric tumors, RTPCR

Introduction

The cancers of infancy and childhood differ biologically and histologically from the adult tumors. There is difference in incidence, etiology, type of tumor, and improved response of tumor by faster and accurate diagnosis. Leukemia is the most common pediatric malignancy, accounting for approximately one-third of all cases, followed in order of decreasing frequency by brain tumors, lymphomas (Hodgkin's disease and non-Hodgkin's lymphoma), neuroblastoma, soft-tissue sarcomas (including rhabdomyosarcoma), kidney tumors (primarily Wilms' tumor), bone tumors (osteosarcoma and Ewing's sarcoma [EWS]), and retinoblastoma.[1-7] During the past 30 years, the prognosis for all types of childhood cancer has improved dramatically. Environmental and genetic factors are linked to an increased risk of cancer. For example, exposure to ionizing radiation, toxic chemicals (such as benzene), and specific antineoplastic drugs are all associated with a higher risk of acute leukemia. Genetic diseases associated with chromosomal instability—including Bloom's syndrome, Fanconi's anemia, and ataxia-telangiectasia—are also characterized by an increased risk of acute leukemia. Similarly, children with congenital immunodeficiencies, such as Wiskott-Aldrich syndrome, are predisposed to the development of leukemia. In addition, Down syndrome, which is not associated with chromosomal instability or immunodeficiency, is associated with a 10-to 20-fold increase in acute leukemia. However, the etiologic basis of the vast majority of cancer cases, especially in children, remains unclear.

Rhabdomyosarcoma is the most common type of pediatric soft tissue sarcoma, comprising about 50% of cases. The contributions of molecular genetics have significantly improved the accuracy of diagnosis within subtypes and enhanced the understanding of oncogenesis. A characteristic translocation, t(2;13)(q35;q14), is seen in about 75% of cases of alveolar rhabdomyosarcoma. With this translocation, the PAX3 gene on chromosome 2 is fused with the FKHR (also known as FOX10A or ALV) gene on chromosome 13, resulting in a chimeric transcription factor derived from the 5′ end of the PAX3 gene and the 3′ end of FKHR gene (exons 2 and 3). Both portions encode DNA-binding domains. Recurrent cytogenetics changes (+2, +7, +8, +11, +12, etc.) have been observed in embryonal rhabdomyosarcoma. EWS and peripheral primitive neuroectodermal tumor (PNET) are associated with a reciprocal translocation involving chromosome 22 at q12. In about 90% of cases, the other involved chromosome is 11 at q24. This translocation fuses the FLI-1 gene (on chromosome 11) with the EWS gene (on chromosome 22), resulting in a chimeric protein derived from the 5′ end of EWS and 3′ end of FLI-1.

Desmoplastic small round cell tumor (DSRCT), the EWS gene on chromosome 22 is fused with the Wilms tumor suppressor gene (WT1) gene on chromosome 11. The resulting chimeric protein is derived from the 5′ end of the EWS gene and the 3′ end of the WT1 gene. As in EWS, the fusion gene in DSRCT includes up to exon 7 and rarely exon 8, 9, or 10 of EWS.

Synovial sarcoma has characteristic translocation t(X;18)(p11;q11) which has been identified in up to 95% of cases of synovial sarcoma. In the vast majority of cases, this translocation leads to a fusion transcript of the 5′ portion of the SYT gene on chromosome 18 with the 3′ portion of one of two genes, SSX1 or SXX2, on chromosome X. Both monophasic (purely mesenchymal morphology) and biphasic

Table 1: Chromosomal translocations in the childhood tumors

Type of tumor	Chromosomal translocation	Genes involved in fusion	Prognosis
Embryonal rhabdomyosarcoma	Gains of 2, 7, 8, 12, 13; losses of 1, 6, 9, 14, and 1750	IGF2, GOK, PTCH TP53	Good
Alveolar rhabdomyosarcoma	t (2;13)(q35;q14); t (1;13)(p36;q14)	PAX3-FKHR PAX7-FKHR	Poor
EWS/PNET	t (11;22)(q24;q12) t (21;22)(q22;q12) t (17;22)(q21;q12) t (2;22)(q33;q12) Inversion of 22q	EWS-FLI-1 EWS-ERG EWS-ETV1 EWS-E1AF EWS-FEV EWS-ZSG	Good with Type I fusion transcripts
DSRCT	t (11;22)(p13;q12)	EWS-WT1	Poor
Clear cell sarcoma	t (12;22)(q13;q12)	EWS-ATF1	Poor
Extraskeletal myxoid chondrosarcoma[§]	t (9;22)(q22–23;q11–12)	EWS-TEC	Good
Synovial sarcoma	t (X; 18)(p11;q11)	SYT-SSX1	Poor
Congenital/infantile-fibrosarcoma	t (12;15)(p13;q25)	ETV6-NTRK3	Good
Inflammatory myofibroblastic tumor	t (1;2)(q25;p23)	TPM3-ALK	Good

EWS: Ewing's sarcoma, PNET: Primitive neuroectodermal tumor, DSRCT: Desmoplastic small round cell tumor

(with both epithelial and mesenchymal features) variants of synovial sarcoma are associated with this translocation. Most synovial sarcomas with SYT/SSX1 show biphasic morphology; in contrast, most synovial sarcomas with SYT/SSX2 show monophasic morphology. Despite the lack of a recognized predisposing genetic condition in the most cases of malignancy, cancer must still be considered a genetic disease. That is, somatically acquired genetic changes play a major role in the pathogenesis of both adult and childhood tumors. Table 1 summarizes the molecular translocations in childhood tumors,[8-11] and Table 2 summarizes the chromosomal translocation in acute leukemias of childhood.

Diagnostic and Clinical Implication of the Molecular Translocation

Among the small round blue cell tumor rhabdomyosarcoma which is treated primarily with chemotherapy. The role of surgery is limited to initial biopsy, wide local excision (whenever clear margins are possible), and resection of residual disease. Radiotherapy in the form of external beam or brachytherapy is restricted to persistent or recurrent disease. The primary treatment for Ewings/PNET is surgical resection, but adjuvant radiotherapy and chemotherapy are also being used. The accurate diagnosis of pediatric tumors is critical for treatment and prognosis. Guideline for handling ambiguous and mismatched biopsy report and molecular genetics results has been suggested by Ladanyi and Bridge.[6] If a gene fusion is absent in cases of tumor type typically having the gene fusion, the quality of molecular diagnostic methods should be further evaluated to avoid false-negative results. In contrast, if a given gene fusion is present by one molecular diagnostic test in cases of tumor type typically not having the gene fusion, other molecular diagnostic tests should be performed to exclude false-positive interpretation.

Table 2: Chromosomal translocation in acute childhood lymphoblastic leukemias

High hyperdiploidy	Favorable
Hypodiploidy	Intermediate for patients with 45 chromosomes Adverse for patients with<45 chromosomes Intermediate for patients with<46 chromosomes
Near-haploidy	Adverse
t (12;21)(p13;q22)/ ETV6-RUNX1 (TEL-AML1)	Favorable
t (9;22)(q34;q11.2)/BCR-ABL1	Adverse
t (4;11)(q21;q23)/MLL-AFF1(AF4)	Adverse
t (1;19)(q23;p13.3)/der(19)t(1;19)	Favorable
(q23;p13.3)/PBX1-TCF3 (E2A)	Intermediate
t (10;14)(q24;q11)/TCRA/ TCRD-TLX1 (HOX11)	Not determined
del (6q)	Not prognostic
Abnormal 9p	Not prognostic Adverse
Abnormal 12p	Not prognostic
Normal karyotype (no aberration detected)	Relatively favorable

Prognostic Applications of Molecular Translocations in Childhood Tumors

Studies have suggested that the different fusion proteins in each specific soft tissue tumor may have prognostic significance. Anderson *et al*.[12] recently reported the translocation, t(2;13)/PAX3-FKHR, to be an adverse prognostic factor for alveolar rhabdomyosarcoma. In contrast, t(1;13)/PAX7-FKHR was associated with a favorable prognosis and was more frequently observed in younger patients with relatively localized disease. Sorensen *et al*.[13] reported that, among the patients with metastatic alveolar rhabdomyosarcoma, bone marrow

involvement was significantly higher in PAX3-FKHR-positive patients. Furthermore, in patients presenting with metastatic disease, there was a striking difference in outcome between PAX7-FKHR and PAX3-FKHR patient groups (estimated 4-year overall survival rate of 75% for PAX7-FKHR versus 8% for PAX3-FKHR).[13]

Similarly, recent studies have suggested that the most common type of EWS-FLI1 fusion transcript, Type 1 (EWS exon 7 fused to FLI-1 exon 6), is associated with a favorable prognosis and appears to encode a functionally weaker transactivator, compared to other types of fusion transcripts.[14,15] SYT-SSX2 fusion variant had significantly longer metastasis-free survival than those with SYT-SSX1 variant.[16] Ladanyi *et al*.[17] reported that the patients with the SYT-SSX2 fusion variant had better overall survival than those with SYT-SSX1 variant.

Conclusion

Molecular diagnostic techniques reverse transcription polymerase chain reaction and fluorescence *in situ* hybridization detecting the chromosomal rearrangements, translocations, and deletions in childhood leukemias, and soft tissue tumors are useful for targeted therapy, prognosis and detecting minimal residual disease.[18-20]

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