総会記録

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Welcome back lecture 1

Chromosomal Translocations in the Diagnosis of Pediatric Soft Tissue Sarcoma

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1. Abstract

Purpose : Pediatric soft tissue sarcomas have many similarity among different tumors. These tumors present small round cell types, and cause frequent diagnostic problems in pediatric pathology. An important advance in the investigation of these small round cell tumors has been the identification of consistent chromosomal translocations associated with several types of tumor.

Method : Thirty six patients with soft tissue sarcoma were available for review. Seventeen cell lines were also included in this study. The RNA from the specimens were analyzed by reverse transcriptase-polymerase chain reaction (RT-PCR).

Results : PAX 3-FKHR fusion was present in six of eight alveolar rhabdomyosarcoma and PAX 7-FKHR fusion was detected in two of eight alveolar rhabdomyosarcoma. None of the specimens expressed more than one chimeric transcript. EWS-FLI 1 or EWS-ERG fusions were detected in all ten Ewing's sarcoma. No specimens showed EWS-WT 1 fusion. These results corresponded well to the histopathologic diagnosis. There were no differences in the histologic appearances of tumors with the more frequent PAX 3-FKHR or EWS-FLI 1 fusions compared with those containing the variant PAX 7-FKHR or EWS-ERG fusions.

Conclusion : RT-PCR assay for chimeric transcript is an useful tool for a rapid and objective diagnosis of pediatric solid tumors. Through these tools, we can approach genetically to the differential diagnosis of undifferentiated small round tumors.

Key words : Chimeric transcript, Rhabdomyosarcoma, Ewing's sarcoma

2. Introduction

Pediatric soft tissue sarcomas have similar pattern of pathology with uniform small round cells. Despite the existence of a widely accepted classification system, the histologic subtyping of rhabdomyosarcoma (RMS) is often complicated by the relatively high rate of disagreement (20 to 30%) among pathologists[1]. A consistent translocation involving chromosomes 2 and 13 t(2 ; 13) (q35 ; q14) has been identified in the majority of alveolar RMS[2]. The characteristic t (2 ; 13) (q35 ; q14) translocation in the alveolar RMS has been reported[3]. The application of chromosomal translocation to distinguish histologic classification has been a major diagnostic advance. Consistent translocations have been also found in Ewing's sarcoma, Wilms' tumor and desmoplastic small round cell tumor[4)-6)]. We tested these choromosomal translocation assays with histopathologic analysis to demonstrate their usefulness in the diagnosis of pediatric soft tissue sarcoma.

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3. Materials and Methods

3.1 Tumor cell lines

We tested 6 RMS (SJRH 3, SJRH 18, RD, A-673, KYM-1, SCMC-RM 2), 4 Ewing's sarcoma (SCMC-ES 1, ES-1-OT, EW-1, T 50), 2 Wilms' tumor (HFWT, YSWT 1), and 5 lymphomas (LBRM-33, P-2003, RA 1, RPMI 6666, EL 4). All the cell lines were cultured in RPMI 1640 containing 10% fetal bovine serum.

3.2 Primary tumor specimens

Six alveolar RMS, 8 embryonal RMS, 6 Ewing's sarcomas, 10 Wilms' tumors, and 6 lymphomas were included in this study. These specimens were taken from the patients at Yonsei Medical Center before chemotherapy. Tissue specimens were immediately frozen in liquid nitrogen and stored at −80℃. Normal control samples included skeletal muscle and thymus gland specimens obtained from non-cancer patients after obtaining the informed consent of the parents. Tumor samples (100 mg) were frozen in liquid nitrogen and pulverized in autoclaved foil envelopes.

3.3 Total RNA isolation

Total RNAs were extracted from the cell lines and tissues using acid guianidinium thiocyanate-phenol chloroform method[7].

3.4 RT-PCR detection of mRNA expression of chimeric transcript

Randomly primed cDNAs were reverse-transcribed from 4 μg of total RNAs using a cDNA synthesis kit (Boehringer Mannheim Corp., Mannheim, Germany) in a 20-μl mixture as described[8]. Two μl of the cDNA conversion mixture was amplified by PCR. The 2-μl mixture was increased to 100 μl by adding 1-mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM of each deoxynucleotide triphosphate, 6 units of Taq polymerase, and 50 pmol of each of the specific oligonucleotide primers for PAX 3-FKHR, PAX 7-FKHR, EWS-FLI 1, EWS-ERG, EWS-WT 1.The sequences of the oligonucleotide primers for RT-PCR are shown in Table 1 and Table 2. β-Actin-specific oligonucleotides provided a quantitative control of the reaction. PCR amplification was performed in a DNA thermal cycler (Perkin-Elmer Corp.) and consisted of 30 or 40 cycles of denaturation for 1 min at 94℃, annealing for 1 min at a temperature appropriate for the various primers, and extension for 2 min at 72℃. A final extension proceeded for 7 min at 72℃. The β-actin gene was amplified at 94℃ for 1 min, and 72℃ for 2 min for 30 cycles. The PCR reaction products were electrophoresed on 2% agarose gels, and stained with ethidium bromide.

Table 1 Oligonucleotide primers used in reverse trascriptase-polymerase chain reaction assays.

Primer	Sequence
5′ PAX3	GCACTGTACACCAAAGCACG
5′ PAX7	TTTGAGAGGACCCACTACCC
5′ FKHR	GCAGATCTACGAGTGGATGG
3′ FKHR	AACTGTGATCCAGGGCTGTC
5′ EWS-A	TCCTACAGCCAAGCTCCAAGTC
5′ EWS-A	TCCTACATGCCAAGCTCCAAG
3′ FLI1	ACTCCCCGTTGGTCCCCTCC
3′ ERG	ACTCCCCGTTGGTGCCTTCC
3′ WTI	CAGCTGGAGTTTGGTCATG
5′ β actin	CTGTCTGGCGGCACCACCAT
3′ β actin	GCAACTAAGTCATCATAGTCCGC

Table 2 Cytogenetic translocations and their corresponding chimeric products.

Translocation	Chimeric product
t(2 ; 13) (q35 ; q14)	PAX3-FKHR
t(1 ; 13) (p36 ; q14)	PAX7-FKHR
t(11 ; 22) (q24 ; q12)	EWS-FLI1
t(21 ; 22) (q22 ; q12)	EWS-ERG
t(11 ; 22) (p13 ; q12)	EWS-WT1

3.5 Histopathologic analysis

All available sections were examined without knowledge of patient identities, original diagnosis, clinical features, or results of chromosomal analysis. Slides from thirty six patients were reviewed by two pediatric pathologist, and in all cases there was agreement in the histopathologic diagnosis.

4. Results

The PAX 3-FKHR chimeric message was detected in two cell lines and four tumor samples of alveolar RMS. The PAX 7-FKHR fusion transcript was

Table 3 RT-PCR and histopathologic results.

RT-PCR	Histopathologic results											
	ARMS		ERMS		Ewing		WT		Lymphoma		Total	
	Cell	Tumor	Cell	Tumor	Cell	Tumor	Cell	Tumor	Cell	Tumor	Cell	Tumor
PAX3-FKHR	2	4	0	0	0	0	0	0	0	0	2	4
PAX7-FKHR	0	2	0	0	0	0	0	0	0	0	0	2
EWS-WT1	0	0	0	0	0	0	0	0	0	0	0	0
EWS-FLI1	0	0	0	0	3	6	0	0	0	0	3	6
EWS-ERG	0	0	0	0	1	0	0	0	0	0	1	0
Negative	0	0	4	8	0	0	2	10	5	6	11	24
Total	2	6	4	8	4	6	2	10	5	6	17	36

Abbreviation : cell, preserved known cell-line ; tumor, tumor specimen from patients, ARMS, alveolar RMS, embryonal RMS ; WT, Wilms' tumor

detected in two tumor samples of alveolar RMS. None of embryonal RMS showed either PAX 3-FKHR or PAX 7-FKHR chimeric transcript. These results confirm that the PAX 3-FKHR and PAX 7-FKHR transcript is specifically associated with the alveolar RMS. We observed no differences in the histologic appearances of tumors with the more frequent PAX 3-FKHR fusions compared wiht those containing the variant PAX 7-FKHR fusions.

A hybrid transcript EWS-FLI 1 was found in 9 of 10 cases of Ewing's sarcoma. Only one cell line of Ewing's sarcoma showed EWS-ERG taranscript. However, none of the cell lines or tumor sample showed EWS-WT 1 transcript. Twenty three cases histopathologically diagnosed as either Wilms' tumors or lymphoma were negative in the five RT-PCR assays (Table 3).

5. Discussion

An important advance in the investigation of these small round cell tumors has been the identification of consistent chromosomal translocations associated with several types of tumor. Chromosomal translocation generating unique chimeric genes are highly chacteristic of specific sarcomas, and their use as diagnostic markers has been suggested[9]. This study was designed to examine the usefulness of molecular assays in soft tissue sarcoma diagnosis ; it does not reflect the overall frequency of these genetic alterations in soft tissue sarcoma. Pediatric soft tissue sarcomas have many similarity among different tumors. These tumors present small round cell types, and cause frequent diagnostic problems in pediatric pathology[10][11].

Alveolar RMS is associated with a characteristic translocation t(2 ; 13) (q35 ; q14)[12]. The genes involved in this translocation are paired box (PAX)3 on chromosome 2 and forkhead in RMS (FKHR) on chromosome 13. An occasional variant translocation t(1 ; 13) (p36 ; q14) affecting PAX 7 and FKHR on chromosomes 1 and 13, respectively, has also been described. Chromosomal translocations in RMS are detected using conventinal cytogenetic analysis, fluorescence in situ hybridization (FISH) or RT-PCR on fresh or frozen tissue samples[2][3][13][14]. PAX 3-FKHR and PAX 7-FKHR are potent transcriptional activators. Gain of function mutations creating fusion proteins consisting of PAX 3 or PAX 7 and another transcription factor of the forkhead family (FKHR) are associated with alveolar RMS. The analyses of PAX 3 and PAX 7 function in normal development indicate that PAX 3 and PAX 7 triggers neoplastic development by maintaining cells in a deregulated undifferentiated and proliferative state in alveolar RMS[15]. The PAX 3-FKHR chimeric transcript was detected in six of eight cases of alveolar RMS, but not in any of the 12 embryonal RMS. The PAX 7-FKHR fusion transcript was detected in two of eight alveolar RMS in this study. These results confirm that the

PAX 3-FKHR and PAX 7-FKHR transcript is specifically associated with the alveolar RMS. The PAX 3-FKHR transcript is specifically associated with the alveolar RMS and that it is a more sensitive marker of the t(2 ; 13) than the reciprocal product FKHR-PAX 3[16].

Ewing's sarcoma is primary malignancy of bone and soft tissues characterized in at least 96% of cases by specific fusion transcripts originating from recurrent chromosomal translocations. Ten cases had histopathologic review diagnosis of Ewing's sarcoma, of which nine had an EWS-FLI 1 fusion, and one had an EWS-ERG fusion in this study. There were no significant clinical differences observed between EWS-ERG fusion and EWS-ELI 1 fusion in age of diagnosis, sex, metastasis at diagnosis, primary site. It is also noticed by the other report, they reported that differences in the C-terminal partner in the Ewing's sarcoma family gene fusions are not associated with significant phenotypic differences[17]. Detection of the chimeric RNA transcripts by RT-PCR has greatly facilitated the diagnosis of Ewing's sarcoma. The proximate role of EWS-FLI 1 in the pathogenesis of Ewing's sarcoma is thought to involve the activation of as yet largely unknown target genes[18]. The suggested combination of RT-PCR and restriction analysis of the PCR products allows a rapid and specific determination of Ewing's sarcoma specific translocations[19)-21].

Two reliable assays for the chimeric transcript in alveolar RMS and Ewing's sarcoma could be established. These molecular diagnostic methods can provide an objective, rapid, and efficient means of making an accurate diagnosis and classification in undifferentiated small round cell tumors.

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