Brief report

Abnormality of chromosome 8 in desmoid-type fibromatosis

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Desmoid-type fibromatoses are clonal fibroblastic cell proliferations that arise in deep soft tissues and are characterized by infiltrative growth and a tendency toward local recurrence. The molecular genetic studies show that there are chromosomal abnormalities relating to the pathogenesis of this lesion, such as trisomy 8. However, there are different results concerning this type of abnormality. Fluorescence in situ hybridization (FISH) is a sensitive and reproducible technique that combines molecular cytogenetics with morphologic information and brings them together in a single frame for evaluation. To explore the controversy of the aneuploid abnormality of chromosome 8, we collected specimens of desmoid-type fibromatosis to determine whether the existence of trisomy 8 chromosome abnormalities correlates with clinicopathologic features, as well as to investigate the feasibility of detecting trisomy 8 in FFPE tissue by FISH method.

METHODS

Patients
From 1994 to 2005, 96 patients in the database of Department of Pathology, Cancer Hospital of Fudan University had a histologically confirmed diagnosis of desmoid-type fibromatosis. We used paraffin blocks from 28 patients. For all the recurrent patients, it was confirmed that the lesions had no correlation with the last surgery by the evidence such as the pathology report, case history and the records of surgery.

Probe
The CEP 8 probe (Vysis, USA) was used. It is a SpectrumOrange™ labeled probe specific for the alpha satellite (centromeric) region, 8p11.1-q11.1. The probe was designed for the detection and quantification of chromosome 8 in both interphase nuclei and metaphase spreads by FISH.

Procedure
Pretreating Slides
Briefly, the paraffin sections were baked and deparaffinized. The dried-tissue sections were immersed in 0.2 mol/L HCl for 20 minutes. After washing, the sections were placed in Pretreatment Solution (1 mol/L NaSCN) at 80℃ for 30 minutes. The sections were covered with prewarmed Protease K (50 μg/ml, Merk, Germany) and incubated at 37℃ for 8-15 minutes.

Denaturation of specimen DNA and hybridization
Denaturation of the specimen DNA were performed in the denaturing solution (70% formamide/2×SSC, PH 7.0) at 73℃ for 5 minutes then air-dried. The probe was pre-denatured and ready to apply to the denatured target area. Total 5-15 μl CEP 8 probe was applied to the tissue sample according to the size of the tissue sections. A glass coverslip (Menzel, Germany) was placed onto the tissue and sealed with rubber cement (Fixogum, Germany). Hybridization was performed on a HyBrite™ instrument (Vysis, USA) at 42℃ for 12-18 hours.

Post-hybridization washes and evaluation of Fish signal
Afterwards, the slides were washed in 0.4×SSC and 2×SSC/0.1% NP-40. The sections were dried and 10 μl of DAPI II was applied. The samples were examined with an Olympus BX-60 fluorescent microscope as per the probe kit instructions. The tumor with more than 2.2% nuclei displaying three fluorescent signals was considered to have an abnormal trisomy 8.

Quality control
Positive control tissue was fixed biological specimens, which derived from a mixture of 10% trisomy 8 and 90% disomy 8 cultured human lymphoblast cells on microscope slides. Negative control tissue was approximately 0% trisomy 8. Staining of experimental slides was accompanied concurrently with positive and negative control slides to monitor assay performance and to assess the accuracy of signal enumeration. No routine FISH test results were reported if the assay controls failed.

Statistic analysis
All statistical analyses were performed with SPSS 11.5 (USA). χ² test and Fisher’s exact tests were used and a significantly different P value was set at 0.05.

RESULTS
There were 6 male and 22 female patients with a median age of 36.8 years (ranging from 14 to 86 years). Among
all desmoid-type fibromatoses, 28.6% (8/28) were recurrent lesions. Patients with recurrent lesions were not significant younger than patients with primary lesions.

Results of FISH of 20 cases could be used for the analysis. As shown in Fig., the specimens of desmoid-type fibromatosis contained cell subpopulations with trisomy 8. Six cases (30%) contained cell subpopulations with trisomy 8, 1 found in the 12 primary lesions and the other 5 in the 8 recurrent lesions (Table). The percentage of trisomy 8 in the recurrent lesions (62.5%, 5/8) was significantly higher than that of primary lesions (8.3%, 1/12). There was a close relation between the trisomy 8 and the recurrent lesions (the Spearman Correlation coefficient is 0.58).

The most important factors responsible for the aneuploidy are failure of the mitotic checkpoint caused by mutation of the responsible genes and telomere dysfunction. In the series of Dal Cin et al, trisomy 8 was found by FISH in two of three recurrent desmoid-type fibromatoses but in only two of seven primary tumors. The same results were reported by Fletcher et al, who suggested that desmoid-type fibromatosis with trisomy 8 might be at increased risk of subsequent recurrence. It will be interesting to know if there is any difference in follow-up between the two cytogenetically different groups of patients with desmoid-type fibromatosis.

In our study, FISH revealed trisomy 8 in desmoid-type fibromatosis from 30% of the patients. There is a close relationship between the trisomy 8 and recurrent lesions. These findings provide further evidence of clonality in desmoid-type fibromatosis and indicate that trisomy 8 might identify a subgroup of desmoid-type fibromatoses at high risk for recurrence, which agrees with the suggestion of Fletcher and Dal Cin et al.

Trisomy 8 has been reported in other fibrosing conditions; including Peyronie's disease, Dupeytre's contracture and carpal tunnel syndrome. Fletcher et al have assumed that trisomy 8 abnormalities may contribute to "a wide spectrum of pathologic fibrous proliferations". However, karyotyping studies failed to demonstrate trisomy 8 in recurrent desmoid-type fibromatoses among a large series described by Bridge et al. There are two possible reasons for this contradiction. One is that the above method has limitations, particularly with sensitivity and selectivity. Interphase FISH is remarkably free of the shortcomings of traditional cytogenetics. Another reason for failure to detect trisomy 8 in desmoid-type fibromatoses might be due to rapid overgrowth of the trisomic cells by diploid populations. This possibility gains support from recent findings of Dal Cin et al. Accordingly, trisomy 8 as a potential prognosticator of recurrence and its significance in desmoid tumors requires further evaluation.

Over the last decade, FISH has emerged as a powerful tool for the analysis of genetic changes in tumors. However, the success rate of FISH in detecting trisomy 8 in desmoid-type fibromatosis is not as high as expected. Failure to detect trisomy 8 in some cases might be due to limitations of the method, such as sensitivity, selectivity, and denaturing temperatures and denaturing methods.

Among the specimens analyzed those from 30% of cases contained cell subpopulations with trisomy 8. Specimens could generate ideal results after successful pretreatment and optimization.

DISCUSSION

Desmoid-type fibromatoses are intermediate (locally aggressive) fibroblastic and myofibroblastic tumors. The disease is characterized by aggressive local infiltration of surrounding tissues. Microscopically, the lesions show longitudinally oriented fascicles of spindled fibroblasts and myofibroblasts in a prominently collagenous background. Wide excision, function-preserving surgery is the goal in treatment of desmoid-type fibromatosis. There is still, however, a high risk of local recurrence after treatment. Recurrence has been difficult to predict using clinical or histologic criteria, and a reliable marker of recurrence could be useful in determining optimal surgical margins or need for adjuvant radiation therapy. A provocative suggestion that trisomy 8 might be associated with an increased likelihood of local recurrence in this tumor was reported. The purpose of this study was to analyze patients with desmoid-type fibromatosis to determine whether trisomy 8 correlated with disease recurrence.

**Table.** Incidence of trisomy 8 in different type desmoid-type fibromatosis

<table>
<thead>
<tr>
<th>Type of desmoid-type fibromatosis (n (%))</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td>Primary lesions</td>
<td>Recurrent lesions</td>
</tr>
<tr>
<td>Trisomy 8 positive</td>
<td>1(8.30%)</td>
</tr>
<tr>
<td>Trisomy 8 negative</td>
<td>11(91.7%)</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
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</table>

Eight specimens were not used for the analysis because the control results were not optimal. One specimen was abandoned because of low signal specificity. Two cases had too weak signals because pretreatment, such as deparaffinization. There were three cases that could not be analyzed because of excessive digestion. The other two cases had almost no signal because of inadequate denaturing temperatures and denaturing methods.

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clinical and research tool for the assessment of target DNA levels within interphase nuclei. The main advantage of this technique is the combination of the detection of cytogenetic aberrations with preserved morphology. In many cases only FFPE tissues are available for analysis, so optimal methods to use these tissues for accurate diagnosis need to be investigated.

In our study, the specimens of FFPE tissues gave ideal results. Among the specimens analyzed, 30% of cases contained cell subpopulations with trisomy 8 and the incidence accorded with the reported results. Eight cases were not ideal because of some inadequate processing. During the processing one of the main steps is the pretreatment of the specimen. During the pretreatment of the tissues in the pretreatment solution temperature, concentration and pH of the solution might affect the assay results. For the archival FFPE tissues, the concentration and pH of the solution might affect the assay results. For the archival FFPE tissues, the pretreatment solution is 1 mol/L NaSCN solution at 80°C for 30 minutes. The other important step is the tissue digestion. It is important to evaluate the degree of protein digestion before moving on to hybridization. We suggested a range of times because some tissues are more resistant to digestion; such as desmoplastic small round cell tumor and desmoid-type fibromatosis in this study. For these tissues proteinase K may work better than pepsin digestion (i.e. proteinase K at 10 mg/ml in 2× SSC, at 37°C for 20 minutes).

There are other techniques reported for the digestion and pretreatment steps. Some consider that 3 minutes of pepsin digestion allowed FISH analysis of all specimens producing a high hybridisation efficiency and well-preserved morphology. Others suggest that the use of pepsin alone is not sufficient for histological sections and microwave pretreatment is mandatory in these cases. Leers et al proposed that heating dewaxed tissue sections leads to a diminishing of the nucleic proteins, allowing propidium iodide to get into the nuclei. This effect could also be helpful for diffusion of probes in the nuclei. However, it is controversial as to whether these pretreatments works. So just like the methods of antigen reparation, different labs have different ways of using the technique and the results are the most important thing.

For the hybridization the HyBrite™ machine was the best choice. HyBrite is a convenient open system for hands-free denaturation and hybridization when using in situ DNA probe procedures. It eliminates a number of steps and reduces the hands-on time required during conventional FISH procedures performed by cytogenetic, pathology and research laboratories.

Over all, from our study we can draw the conclusion that with the adequate and optimal pretreatment work, the FFPE tissue specimens can be used to generate ideal results with bright and specific signals. With this protocol, the advantages of FISH can be used not only for research but also for clinicopathologic diagnosis, which is important for the pathologists when facing difficult diagnostic questions.

REFERENCES


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