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Advances in Brief

Aberrant FHIT Transcripts in Merkel Cell Carcinoma[1]

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Abstract

Merkel cell carcinoma is a rare neuroendocrine carcinoma of the skin which shares several features with small cell lung carcinoma. In a previous study, we reported a high frequency of abnormalities of the *FHIT* gene, located at 3p14.2, in small cell lung tumors. To determine the role of the *FHIT* gene in small cell neuroendocrine malignancies, 14 cases of Merkel cell carcinoma were analyzed by reverse transcription of FHIT mRNA followed by PCR amplification and sequencing of products. Eight of 14 tumors (57%) displayed abnormal FHIT products that lacked three or more exons of the *FHIT* gene. The pattern of abnormal transcripts was similar to that observed in small cell lung tumors, suggesting that FHIT abnormalities might be a common genetic marker of these two types of neuroendocrine tumors.

Introduction

MCC[3] is an uncommon tumor of controversial origin first described by Toker with the name of trabecular carcinoma (1, 2). MCC shares common features with SCLC, including morphological and immunophenotypical characteristics as well as some aspects of natural history (3). In fact, both of these small cell tumor types have neuroendocrine features and express specific neuropeptides and intermediate filament protein (2). Tumor cell lines established from MCC show lack of substrate adhesion and grow as floating aggregates, a pattern observed in the majority of SCLC cell lines (4, 5). Clinically, both MCC and SCLC show a high incidence of recurrences and nodal metastases, although MCC behaves less aggressively (6–8).

In contrast to SCLC, in which deletions of the short arm of chromosome 3 (3p) are observed in a majority of the patients (9, 10), cytogenetic studies of MCC tumors and cell lines did not show 3p involvement (4, 11, 12–15). However, a study by Leonard *et al.* (16) of 26 MCC tumors revealed loss of heterozygosity at 3p13–21.1 in 69% of the cases, and rearrangement of chromosome 3 was detected in a cell line derived from one tumor by fluorescence *in situ* hybridization.

The *FHIT* gene, recently identified at 3p14.2 (17), has interesting features because it contains the FRA3B fragile site and the breakpoint of the t(3;8) translocation of familial renal cell carcinoma, and is also the target of homozygous deletions in various human cancer cell lines (17, 18). In addition to the first report of aberrant FHIT transcripts in ~50% of gastrointestinal tumors (17), we have recently detected abnormalities of FHIT transcripts in 80% of SCLCs and in at least 40% of non-small cell tumors of the lung (19). The concomitant loss of one *FHIT* allele in the majority of these patients and the genomic rearrangements of the remaining allele in some tumors indicate that the *FHIT* gene is an important target of deletions affecting the short arm of chromosome 3 in lung tumors.

Because of the similarities between SCLC and MCC tumors, we examined 14 cases of MCCs for the presence of FHIT abnormalities by reverse transcription of FHIT mRNA followed by PCR amplification and sequencing of the products.

Materials and Methods

Patients. Fourteen resected tumor samples were obtained from 13 patients at Istituto Nazionale Tumori (Milan, Italy); the samples included 4 primary tumors, 4 recurrences, and 6 nodal metastases. In one patient, we examined both a tumor recurrence and a nodal metastasis (Fig. 1, *Lanes 4* and *5*). The tumors were classified histologically according to Silva's modified criteria and the diagnoses confirmed by agreed immunophenotypical requirements (20). Pathologic staging (pTNM) was carried out according to the tumors-nodes-metastases classification of malignant tumors defined by the International Union Against Cancer (1987). Eight patients were males, and five were females; the mean age of cases at resection was 62 years.

RNA Extraction and Reverse Transcription. Tumor specimens were frozen immediately after surgical resection and stored at −80°C. Total mRNA was extracted from frozen tumor using the RNA-STAT kit (Tel TEST, Inc.). cDNA was synthesized from 1 μg of total RNA. Reverse transcription was performed in a 20-μl volume of 1× first strand buffer (Life Technologies, Inc.). 10 mM DTT (Life Technologies, Inc.), 500 μM dNTPs, 50 ng/μl oligo-dT, 0.3 μg/μl random primers, 16.5 U RNAsin (Promega, Madison, WI), and 300 units Superscript II (Life Technologies, Inc.). The samples were first denatured for 5 min at 95°C and incubated at 37°C for 60 min. The reaction was stopped by inactivating the enzyme at 94°C for 5 min. The reaction was diluted to 30 μl and 1 μl was used for subsequent PCR amplification.

RT-PCR and cDNA Sequencing. 1 μl of cDNA was used for a first PCR amplification in a volume of 25 μl containing 0.8 μM of primers 5U2 and 3D2 (17), 50 μM of each dNTP (TAKARA), 1× PCR buffer, and 1.25 units Taq Polymerase (TAKARA). The PCR consisted of an initial denaturation at 95°C for 3 min and 25 cycles of 15 s at 94°C, 30 s at 62°C, 45 s at 72°C, and a final extension of 5 min at 72°C, using a Perkin Elmer Cetus PCR Thermocycler. The amplified product was diluted 20-fold in TE buffer (1× = 10 mM Tris-HCl, 1 mM EDTA), and 1 μl of the diluted reaction product was subjected to a second round of PCR amplification using nested primers 5U1 and 3D1 (17) for 30 cycles under the above conditions. The PCR products were resolved on 1.5% ethidium bromide-stained Metaphor gels (FMC Bioproducts). Bands were cut from gels, and DNA was purified using QIA quick gel extraction kit (QIAGEN). Depending on the size of the PCR products, 5–50 ng of cDNA were sequenced, using primers 5U1 and 3D1, by the dideoxynucleotide termination reaction chemistry for sequence analysis on the Applied Biosystems models 373A and 377 automated DNA sequencers.

Results and Discussion

To study abnormalities in FHIT transcripts we reverse transcribed mRNA and amplified the cDNAs by nested PCR. Of the 14 specimens analyzed by RT-PCR, 8 (57%) revealed the occurrence of aberrant

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[3] The abbreviations used are: MCC, Merkel cell carcinoma; SCLC, small cell lung cancer; RT-PCR, reverse transcription-PCR.

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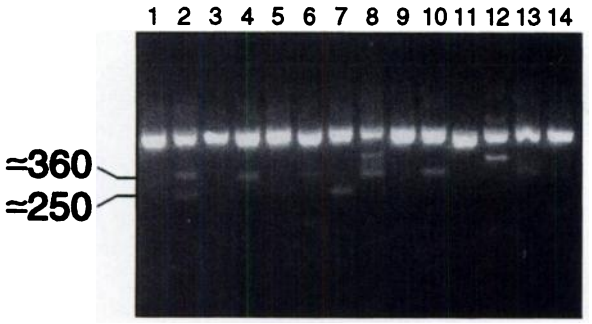

Fig. 1. Expression of the *FHIT* gene by nested RT-PCR analysis in MCC. Sizes of the aberrant products are shown at the *left*.

transcripts (Fig. 1). In one patient, a tumor recurrence and a nodal metastasis were examined (Fig. 1, *Samples 4* and *5*).

The amplified products from these specimens consisted of one abnormal band in four samples (4, 7, 10, 13) and of two abnormal bands in four samples (2, 8, 12, 14), as shown in Fig. 1. In samples 4 and 5, a recurrence and a nodal metastasis from the same patient, a band of the same size was detected in both specimens, although it appeared less intense in the lymph-nodal metastasis. A normal-sized transcript was present in all cases and probably reflects the presence of normal cells infiltrating the tumor specimen as postulated previously for lung cancer (19).

Abnormal transcripts of either ~360 or ~250 bp or both occurred in six of the eight samples (cases 2, 4, 7, 8, 10, 13, and 14), whereas in two specimens (cases 8 and 12) a product of ~500 and ~450 bp was found, respectively (Fig. 1).

Sequence analysis of normal and abnormal size bands showed that

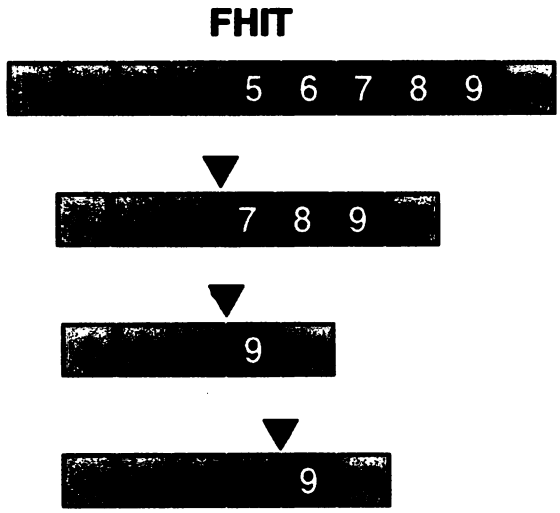

Fig. 2. A schematic representation of the most frequent aberrant transcripts detected in MCC specimens. The coding exons of the *FHIT* gene are in dark colors. *Numbers*, FHIT exons; *arrows*, abnormal junctions between exons 3 and 7, exons 3 and 9, and exons 4 and 9.

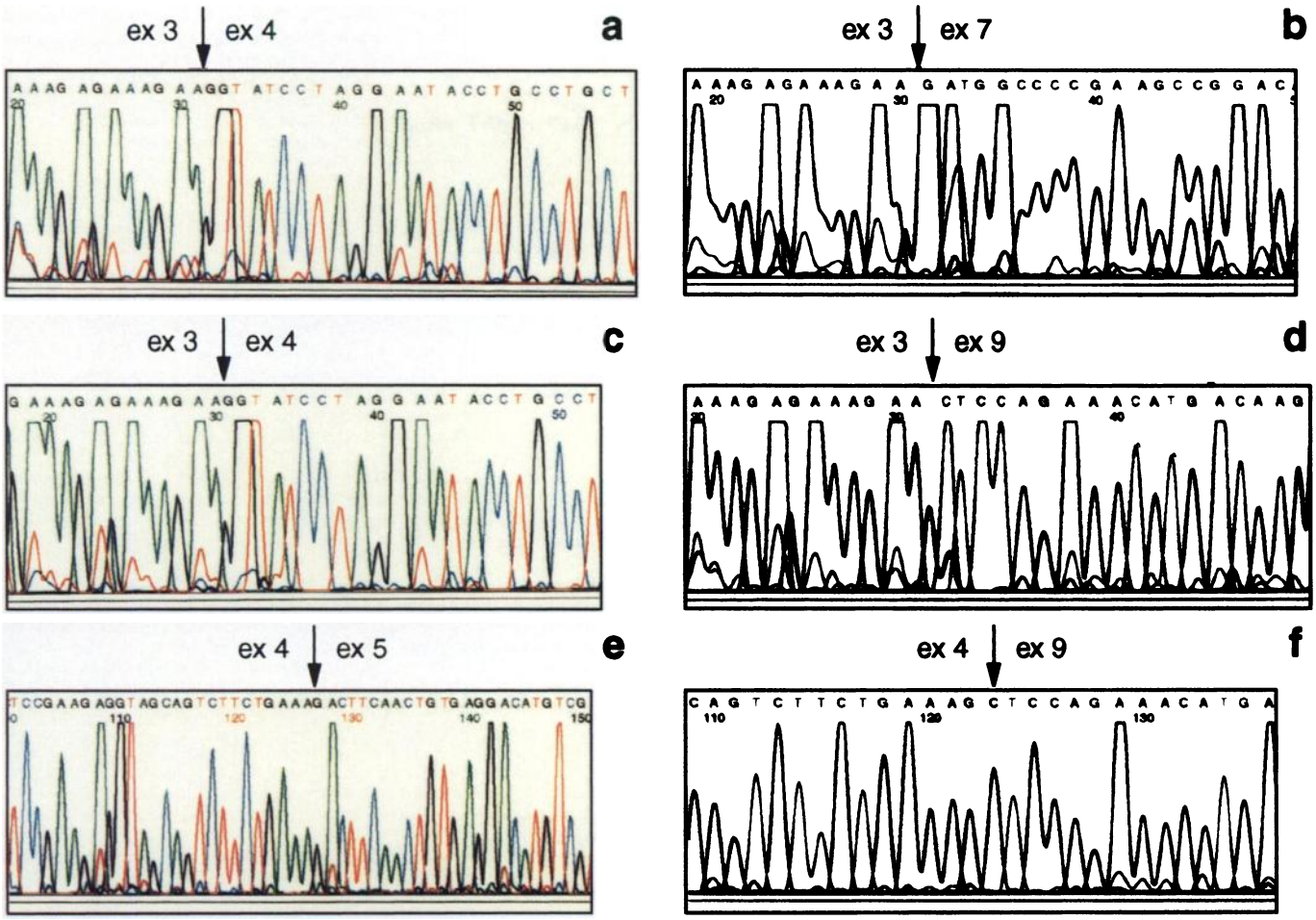

Fig. 3. Sequences of the abnormal transcripts observed in MCC. *Arrows*, junctions between exons 3 and 4 in the wild-type products (*a* and *c*) and junctions between exons 3 and 7 and exons 3 and 9 observed in the abnormal transcripts of cases 2, 4, 7, 8, 10, and 13 (*b* and *d*). *e* and *f*, junctions between exons 4 and 5 in the wild-type product and of exons 4 and 9 in the aberrant transcript of case 8.

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the 360 bp abnormal product corresponded to absence of exons 4 to 6 (nucleotides −111 to 249) of the published FHIT cDNA sequence (17) and resulted in fusion of exons 3 and 7, whereas the 250 bp transcript exhibited loss of exons 4 to 8 (nucleotides −111 to 348), creating a junction between exons 3 and 9 (Figs. 2 and 3, *a–d*).

In sample 2, besides the presence of the 250 bp transcript corresponding to the fusion of exons 3 and 9, a 380-bp product was observed, and sequence analysis showed a loss of exons 4–6, resulting in a junction of exons 3 and 7, with insertion upstream of exon 7 of an 18-bp sequence without significant homology to other sequences.

Analysis of the second abnormal transcript in sample 8 revealed a loss of exons 5–8 (nucleotides −17 to 348) of the FHIT cDNA sequence (Figs. 2 and 3, *e* and *f*). In sample 12, the abnormal transcript was a fusion of exons 3 and 8 with an insertion upstream of exon 8 of a 160-bp Alu sequence. In sample 14, a junction between exons 4 and 8 (nucleotides −17 to −279) of the FHIT cDNA was found in the aberrant product of ~350 bp, resulting in the loss of exons 5–7; in addition, an insertion of a sequence of ~70 bp with no significant homology to other genes was observed at the splice site between exon 4 and 8. In all abnormal transcripts, the fusion junctions coincided with splice sites. Sequence analysis of the normal-sized bands revealed that they contained the normal FHIT cDNA.

This study reports the presence of abnormalities in transcripts of the *FHIT* gene, located at 3p14.2, in approximately 60% of MCC. Besides the normal sized transcript, which is believed to be caused by normal stromal contamination [as suggested by the presence of partial allelic losses (16), as well as by histopathological analyses of this tumor type (6)], the aberrant products observed in these tumors lacked a variable number of exons of the *FHIT* gene, including exon 5, which contains the initial methionine codon and exon 8 where the HIT domain of the gene resides. It is unlikely that the aberrant transcripts encode functional proteins. In six of eight tumors showing FHIT abnormalities, the abnormal transcripts were fusions of exons 3–7 and exons 3–9. These aberrant products corresponded to the type I and II abnormal transcripts, which were consistently found in SCLCs (19).

The results are of interest because both MCC and SCLC share morphological and immunophenotypical features and present some similar behavioral features, such as a high incidence of recurrences and nodal involvement. The less ominous clinical behavior of MCC may be ascribed to the different tissue context of the committed cell to the obvious earlier diagnosis. In fact, about 60% of MCC show recurrences, and 50% have regional nodal metastases, but detailed analysis in terms of pTNM shows for localized, regional, and extraregional disease a tumor death rate of 0%, 11%, and 100%, respectively (6, 8). Similar FHIT transcripts were detected in 80% of SCLCs and about 60% of MCCs, suggesting that similar DNA alterations may have occurred in the two cell types.

The occurrence in MCC of abnormal FHIT transcripts, coupled with the high frequency (69%) of loss of heterozygosity reported in the region 3p13–p21.1 (16), suggests that *FHIT* gene inactivation could be achieved by loss of one allele and rearrangement of the remaining one, as is hypothesized for lung cancer.

The similarity of the human *FHIT* with the *S. pombe* gene homolog suggests that the Fhit protein may have hydrolase activity and thus might cleave the diadenosine 5′,5″-P1,P4-tetraphosphate (Ap4A), a molecule involved in DNA replication and cell cycle control (21, 22). In this context, abnormal FHIT function could have a crucial effect on cell proliferation.

The presence of a fragile site within the *FHIT* gene makes it more susceptible to the breakage caused by chemical and physical carcinogens. SCLCs are linked to carcinogenic exposures to agents such as those found in tobacco smoke; thus, it is of interest that, based on the high predominance of involvement of the head and neck region and the not infrequent coexistence of MCC and basal or squamous cell carcinomas of the skin, UV-light carcinogenesis has been postulated in the etiopathogenesis of at least some Merkel cell tumors (7, 20, 23).

Taken together, the findings of a recurrent pattern of FHIT abnormalities in small cell carcinomas of the lung and of the skin suggest that the *FHIT* gene could be damaged by chemical or physical agents. Ongoing studies are focusing on the cloning and sequencing of the specific breakpoints in these tumor types to understand the molecular basis of FHIT fragility and its involvement in human cancer.

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