Heat Shock Cognate 70 Mutations in Sporadic Breast Carcinoma

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Abstract

Heat Shock Cognate 70 (HSC70) is a constitutively expressed molecular chaperone, functions of which regulate the structure, subcellular localization, and turnover of cell proteins. It is also a component of the centrosome facilitating rearrangements during mitotic/meiotic spindle formation and cytoplasmic microtubule organization. We localized HSC70 to 11q23.3, a region deleted in 40% of sporadic breast and other cancers. Sequencing demonstrated mutation in the NH2-terminal ATPase domain of HSC70 in 2 of 15 sporadic breast carcinomas examined. In both cases, mutation was coincident with allelic imbalance, suggesting that HSC70 is a target of somatic mutation and deletion in a fraction of breast carcinomas.

Introduction

HSC70 is a constitutive member of the highly conserved heat shock protein 70 family, which generally comprises ~1% of total cellular protein with possibly higher levels in transformed cells (1). HSC70 has several known functions: it is the uncoating ATPase for the disassembly of clathrin-coated vesicles (involved in intracellular receptor mediated transport; Ref. 2); it is a molecular chaperone facilitating correct folding of nascent polypeptides (3); it enables protein translocation through the endoplasmic reticulum (Ref. 4; steroid hormone activation), nuclear membrane (5), and into the 26S proteasome (Ref. 6; ubiquitin-mediated degradation); and it is an essential component of the centrosome, facilitating rearrangements during mitotic/meiotic spindle formation and cytoplasmic microtubule organization (7). HSC70 has previously been located to chromosome 11q23.3–q25 (8). AI has been demonstrated at 11q22–q23.1, 11q23.3, and 11q25–qter in malignancy have remained anonymous. ATM and HSC70 have been identified in T-prolymphocytic leukemia (15) or malignant somatic mutations in 11q23.3–q25 (8). AI has been demonstrated at 11q22–q23.1, a region deleted in 40% of sporadic breast and other cancers.

Materials and Methods

Library Hybridization. A contiguous chromosome 11q22–qter YAC library mapped to 11q by STS content was plated in an ordered array. Filters were hybridized sequentially to [32P]ATP. PCR products were resolved on 6% denaturing urea-polyacrylamide gels, and DNA fragments were excised and purified (QIA Quick kit; QIAGEN). The cycle was sequenced using Amplicycle (Perkin-Elmer). Sequence changes were detected in the 15 breast carcinomas. Both of the cases in which mutation had been detected showed AI at 13 breast carcinomas with either one or both markers. Both of the cases in which mutation had been detected showed AI at 13 breast carcinomas with either one or both markers.
rather than T at 3448 in intron 6, and CC rather than AA at 3795, 3796 in intron 7. The deviation in intron 6 is within the sequence of a U14 small nucleolar RNA required for the processing of eukaryotic ribosomal RNA precursors. The change to C gives the human and mouse U14 small nucleolar RNAs identity at this nucleotide (16).

**Discussion**

We have localized HSC70 between D11S1336 and D11S1284 at 11q23.3, a deleted region previously delineated from that at 11q22–23.1 in sporadic breast carcinoma (11, 12). HSC70 is <5 Mb distal to D11S528, with which AI has been reported in 40% of breast carcinomas (12). Our demonstration of AI in 5 of 13 breast carcinomas at D11S1336 and/or D11S1284 corroborates this figure. Sequencing identified mutation in HSC70 in 2 of 15 breast carcinomas, and these mutations were coincident with AI at D11S1336. We therefore propose that HSC70 mutation and deletion are associated with a fraction of sporadic breast carcinomas. Allele loss at 11q23.3 has been identified in a wide variety of solid tumors other than breast (cervical, ovarian, gastric, lung, bladder, prostate, nasopharyngeal, squamous and colorectal cancers, malignant melanoma, and intracerebral neoplasms) at frequencies ranging from 40 to 75% (reviewed in Ref. 21). HSC70 mutation may, therefore, be more generally associated with malignancy.

Of the 15 patients, 3 were homozygous for a 2-bp germ-line deletion adjacent to the splice acceptor sequence in intron 2. This included both the breast carcinomas with mutated HSC70 which, although the number of patients is very small, suggests that there may rather than T at 3448 in intron 6, and CC rather than AA at 3795, 3796 in intron 7. The deviation in intron 6 is within the sequence of a U14 small nucleolar RNA required for the processing of eukaryotic ribosomal RNA precursors. The change to C gives the human and mouse U14 small nucleolar RNAs identity at this nucleotide (16).

hsc70 sequences have been derived from malignant cell line sourced cDNA clones of mouse (F9 teratocarcinoma; Ref. 17) and rat (PC12 pheochromocytoma; Ref. 18). These hsc70 sequences are reported to deviate from hsc70 sequences subsequently derived from nonmalignant tissue of the mouse (19) and rat (20) by a single amino acid. We reasoned that hsc70 could be mutated in the malignant in the cell lines. Analysis of published hsc70 nucleic acid sequences failed to identify codon changes resulting in amino acid deviations between the two genes in each species. Rather, a translation error at codon 97 in exon 3 was suspected in the F9 sequence (GAT = Asn rather than GAT = Asp). We have subsequently sequenced hsc70 from the F9 cell line (genomic DNA and cDNA) and parental 129SvJ6 mouse (genomic DNA) and have confirmed the reported hsc70 sequence (19). No amino acid deviations have been identified between the two rat sequences.

Fig. 1. A, ideogram of chromosome 11q. Microsatellites in the 11q22–qter region are shown (not to scale). Microsatellite loci along the YACs to which HSC70 was localized are shown in greater detail. The position of HSC70 is indicated. B, HSC70 gene structure. Solid horizontal boxes, coding exons. The sequence of the gene from nucleotide 1532 in intron 2 to nucleotide 1708 in the second coding exon is shown. The point mutations in cases 1 and 4 are marked above the sequence, and the nucleotides in the polymorphism are indicated as XX.

Fig. 2. A, sequence analysis of six breast carcinoma samples. The reactions run through the second coding exon initiated from a sequence within intron 3 and are loaded ACGT. The point mutations are indicated. B, microsatellite analysis of four paired normal (N) and breast carcinoma (T) DNA samples. The four cases shown are polymorphic for D11S1336. AIs in cases 1 and 4 are indicated.
be an association between the polymorphism and somatic mutation of HSC70. The polymorphism is noteworthy because of the remarkably repetitive nature of the eight coding exons and seven intervening introns in HSC70 (Fig. 1B), the only heat shock protein 70 family member containing introns. Five of the exons in the human gene encode peptides between 61 and 69 amino acids (including 2 and 3), whereas the remaining three encode peptides of 51, 78, and 185 amino acids (22). Similarly, three of the intervening introns are 322–331 bp (including the first and second), whereas the others are 83, 211, 228, and 249 bp. The conservation of this repetitive gene structure suggests that it is functionally important and that the germ-line deletion, adjacent to the splice acceptor consensus sequence, may itself be corrupting. Alternatively, the polymorphism may be linked to some other sequence that confers some susceptibility to somatic mutation in HSC70. It is, therefore, interesting that the microsatellite distal to the gene was not polymorphic in the three patients homozygous for the 2-bp deletion, whereas that proximal was polymorphic in both of the patients with mutated HSC70.

The two mutations detected in HSC70 were missense mutations in the M44,000 NH2-terminal ATPase domain of HSC70 which, regulated by two cofactors, controls substrate binding by the COOH-terminal of the protein (23). The mutations identified may affect the binding and hydrolysis of ATP and/or the binding of the two cofactors, p48 (the human Hip homologue) and BAG-1. Hip interacts with the ATPase domain of HSC70, following an initial activation by HSP40 stabilizing the ADP-bound form of HSC70, and may prolong its interaction with substrate proteins (24). Hip has also been found to assemble with the progestosterone receptor and as part of an HSC70/HSP90 chaperone complex (Ref. 24 and references therein). Because steroid hormone receptors require sequential interaction with HSC70 and HSP90 to attain their high affinity conformation for hormone binding, Hip-mediated regulation of HSC70 may be required for the assembly of HSP90/steroid hormone receptor complexes. BAG-1, in contrast to Hip, promotes the dissociation of ADP from HSC70, thereby stimulating its ATPase activity (25). Overexpression of BAG-1 prolongs the survival of fibroblast cells challenged by apoptotic stimuli (25). BAG-1 also binds Bcl-2, increasing the antiapoptotic activity of Bcl-2, interacts with hepatocyte and platelet-derived growth factors, enhancing their antiapoptotic activity, and binds and activates the apoptotic protein BAG-1. EMBO J., 16: 3431–3437, 1997.


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