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FHIT

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Inactivation of the *FHIT* gene, encoded at 3p14.2, is one of the earliest and most frequent genetic changes in the development of human cancers. Reexpression of Fhit protein in cancer cell lines with *FHIT* deletions suppresses tumor formation by inducing apoptosis. The 1.5 Mb *FHIT* gene spans the FRA3B fragile site and is prone to deletions, accounting for frequent losses in carcinogen-exposed tissues. Fhit protein and its homologs in animals and fungi form a branch of the histidine triad superfamily of nucleotide binding proteins whose members possess diadenosine polyphosphate (Ap_nA) binding and hydrolysis activity. Fhit dimers bind two Ap_nA substrates in a manner that fills a deep, positively charged groove with substrate phosphates. Available evidence suggests that Ap_nA is a cofactor for the proapoptotic tumor-suppressing function of Fhit.

THE *FHIT* GENE IN NORMAL AND CANCER CELLS

Clear cell renal carcinoma, normally a disease of the elderly, occurs in young adults among carriers of a chromosomal translocation between the short arm of chromosome 3 and the long arm of chromosome 8. The t(3;8)(p14.2;q24) translocation occurs between the third and fourth exons of the *FHIT* gene (1). The FRA3B fragile site, as located by aphidicolin-induced breaks, is located within a 300-kbp region that begins 5′ of exon four and extends through exon five. In cancer cells, the *FHIT* gene frequently contains internal deletions that have removed coding exons and have been repaired by recombination between intronic LINE1 elements (2). In some cases, each *FHIT* allele has lost different exons and the common region of loss is within an intron. In cervical cancer, the fragile region of the *FHIT* gene is targeted by papilloma virus insertion (3). The primary manner by which *FHIT* is inactivated, that is, by deletions rather than point mutations, is similar to that of tumor-suppressor genes such as *CDKN2A* and is dissimilar to that of genes such as *TP53* that are frequent targets of missense mutations. Because *FHIT* has a 441 nucleotide coding sequence within a 1,500,000 nucleotide gene spanning FRA3B, selective pressure for defects in apoptosis is more often resolved by deletions in *FHIT* than by nucleotide substitutions.

TUMOR SUPPRESSION AND CELL DEATH ON REEXPRESSION OF FHIT PROTEIN

Lung (4), stomach, kidney, and gastric (5) cell lines containing *FHIT* deletions, stably transfected with retroviral *FHIT* constructs or infected with adenoviral (6) *FHIT* constructs show suppression of tumorigenesis concomitant with induction of apoptosis. Because lung epithelia are exposed to carcinogens and the *FHIT* locus is fragile, inactivation of *FHIT* in preneoplastic lesions may allow survival of damaged cells and allow cancer progression in response to further genetic changes. Thus, strategies that kill *FHIT* cells before they become malignant might greatly reduce cancer incidence.

Fhit KNOCKOUT MICE

The murine *Fhit* gene, encoded at 14A2, a location syntenic with human 3p14.2, also spans a fragile site (7,8). An embryonic stem cell was subjected to targeted disruption of *Fhit* and animals heterozygous for germline *Fhit* inactivation were obtained. When subjected to intragastric doses of nitrosomethylbenzamine, heterozygous animals developed stomach and sebaceous tumors that resembled human Muir-Torre syndrome (9), a familial cancer syndrome thought to result from deficiencies in mismatch repair. These observations suggest that maintenance of the *Fhit* gene or Fhit protein expression may depend on an intact mismatch repair system.

Fhit ENZYMOLOGY AND PROTEIN STRUCTURE AND RELATIONSHIP TO CELLULAR FUNCTION

Fhit protein is a dimer with two identical binding sites for Ap_nA (10), a family of low-abundance nucleotides that accumulate in response to interferon and contact inhibition of growth. Fhit protein binds ApppA and AppppA with nearly equal affinity (11). The His96Asn allele of Fhit, which binds Ap_nA well but is greatly defective in hydrolysis, is functional in tumor suppression (5,12). Thus, the Fhit-Ap_nA complex is hypothesized to be the active signaling form. Nucleotide hydrolysis and product release may terminate the ability of Fhit to form a complex with a proapoptotic effector. The crystal structure of Fhit bound to nonhydrolyzable ApppA, solved at 2.6 Å resolution, showed that binding of ApnA substrates converts a concave, positively charged surface in the Fhit dimer to a convex surface filled with nucleotide phosphates (10).

Fhit INTERACTING PROTEINS

Although the effectors for the proapoptotic activity of Fhit are not known, it has been observed that Fhit homologs in invertebrates are fused to members of the nitrilase superfamily (13). The worm NitFhit crystal structure shows

that a Nit tetramer binds two Fhit dimers (14). In mammals, Nit and Fhit are not fused but show a highly similar pattern of tissue-specific mRNA accumulation (13). Unlike p53 and many characterized cell death molecules, Nit and Fhit homologs exist in fungi, suggesting that the Fhit pathway predates metazoan evolution (14).

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ADDITIONAL READING

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FIBRILLINS

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Fibrillins1 and 2 (fib1 and fib2) are large extracellular glycoproteins and the core components of microfibrillar aggregates with average diameter of 10 nm. Fibrillin-rich microfibrils are responsible for the biomechanical properties of a wide variety of elastic and nonelastic tissues, either by themselves or together with elastin in the elastic fibers. Fib1 was originally purified from the conditioned medium of cultured human fibroblasts using monoclonal antibodies raised against a microfibrillar extract. Fib2 was serendipitously discovered a few years later during the cloning of the *fib1* gene. The fibrillin proteins display superimposable structures made of tandemly repeated cysteine-rich motifs. Likewise, the fibrillin genes (*FBN1* and *FBN2*) reside on different chromosomes but are similarly organized into a repeated series of exons, each coding for individual cysteine-rich motifs. The bulk of fib1 synthesis mostly correlates with embryonic and postnatal growth, whereas fib2 production peaks earlier and usually before overt elastogenesis. Mutations of FBN1 and FBN2 cause Marfan syndrome (MFS) and congenital contractural arachnodactyly (CCA), respectively. MFS is an autosomal dominant disorder affecting multiple systems and principally, the cardiovascular, ocular, and musculoskeletal systems. CCA is an autosomal dominant disorder akin to, but less severe and more rare than MFS, having predominant manifestations in the musculoskeletal system. The MFS phenotype has been recently replicated in the mouse using the technique of homologous gene targeting in embryonic stem (ES) cells. The study of human and murine mutations has delineated the mechanism of MFS pathogenesis and the role of fibrillin-rich microfibrils in tissue homeostasis. For recent reviews on the fibrillins and their associated pathologies see References (1–3).

STRUCTURE

The modular structure of the fibrillin proteins consists of numerous and tandemly repeated cysteine-rich modules, which are predicted to fold independently. They include: 47 epidermal growth factor-like (EGF) repeats, 43 of which are of the calcium-binding type (cbEGF); 7 repeats containing 8 cysteines (8cys/TB) and common only to the latent TGFβ-binding protein (LTBP); two cysteine-rich sequences unique to fibrillins (Fb) and likely to have arose from the fusion of EGF and TB motifs.

The EGF motif folds into a central β-hairpin and a minor β-sheet at the C-terminus stabilized by disulfide bonds between cysteines 1 to 3, 2 to 4, and 5 to 6. The cbEGF motif contains an additional amino acid pattern (D, D/N,N*, and Y/F, where * denotes a potential β-hydroxylation site) that mediates calcium binding in the N-terminal pocket of the motif (Fig. 1). Unlike the relatively low affinity of isolated cbEGF motifs, tandemly repeated cbEGFs have substantially higher affinities to calcium, either because of local stabilization by adjacent modules or because of longer range effect by a whole series of repeats (4). Apart from stabilizing the linearly rigid structure of fibrillins, calcium binding is believed to mediate monomer interactions, to protect them from extracellular proteolysis, to favor lateral packing of microfibrils, and to organize the macroaggregates.

The 8cys/TB module folds into a globular structure made of six antiparallel β-strands and two α-helices stabilized by disulfide bonds between cysteines 1 to 3, 2 to 6, 4 to 7, and 5 to 8, and containing three consecutive cysteines in the hydrophobic core (Fig. 1). 8cys/TB modules may define the boundaries of functionally distinct and/or coordinately folding series of cbEGF repeats; they may also participate in inter- and intramolecular interactions. The RGD tripeptide located in comparable 8cys/TB motifs of fib1 and fib2 is in a sequence context that predicts interaction with the surrounding cells, probably via the $\alpha v\beta 3$ integrin (5).

Additional fibrillin domains include short cysteine-free segments rich either in prolines (fib1) or in glycines (fib2) and similarly positioned within the N-terminal, third of the