FHIT alterations in breast cancer

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The FHIT gene encodes a diadenosine hydrolase and may be involved in growth control pathways of the cell. Studies on protein–protein interactions, cell lines, including tumourigenicity tests, and knockout mice suggest that the Fhit protein is involved in cell proliferation and apoptosis, and might act as a tumour suppressor. In several different cancers, including breast cancer, alterations in the FHIT gene have been detected in high frequency. The most common alterations are: deletions, DNA hypermethylation, abnormal transcripts and reduced expression at RNA and protein level. The FHIT gene is located at the FRA3B fragile site at chromosome 3p14.2, and alterations in the FHIT gene and Fhit protein have been found associated with genome instability, particularly in BRCA2 mutated breast tumours.

This paper will focus on some of the functional aspects of the Fhit protein with respect to tumour pathogenesis and on aberrations detected in breast cancer.

Key words: breast cancer / FHIT / genetic instability / BRCA2 / deletion / altered expression

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Introduction

At the somatic level only a few genes are found mutated in breast tumours, although genomic instability is detected at a high frequency. Losses of several chromosome regions are recurrently reported, as are amplifications, though to a lesser degree. The amplified regions include well characterized oncogenes, like MYC, ERBB2 and CCND1, but amplifications are only found in a minority of tumours (15–30% for each gene) (reviewed by Ingvarsson, 1999). Similar, mutations in well defined tumour suppressor genes (TSGs) are relatively rare in breast cancer; TP53 is mutated in about 20–30% of breast tumours and to a much lesser degree CDH1, which is found mutated in 56% of lobular breast cancer. Recently, several publications have demonstrated abnormalities at the FHIT (fragile histidine triad) locus in considerably high frequency in either primary breast tumours or cell lines. These include loss of heterozygosity (LOH), as demonstrated using intragenic microsatellite markers, homozygous deletions, hypermethylation of the promotor region, abnormally sized transcripts, and reduced RNA and protein expression. However, no mutations have yet been reported in the FHIT gene in breast cancer cells.

The FHIT gene and Fhit protein

The FHIT gene is located at the FRA3B site of chromosome 3p14.2, and is so far the only example of a gene located in a constitutive fragile region. The FHIT gene is composed of 10 exons which span a 1.8 Mb genome region of which only exons 5–9 are protein coding (Figure 1). Despite spanning a large genomic region, the FHIT gene encodes a small mRNA of 1.1 kb and a small protein of 16.8 kDa. Alternative splicing of the FHIT pre-mRNA has been suggested, but definite characterization of different splicing products in normal cells has not been worked out and so far no alternative proteins have been described.

The yeast homologue of FHIT is the enzyme diadenosine tetraphosphate (Ap(4)A) hydrolase. In higher eukaryotes Fhit may reduce the intracellular level of diadenosine triphosphate, by binding to it and inducing its hydrolysis. A putative role for diadenosine triphosphate in the growth control of the cell has been suggested. Murphy et al. showed that Fhit metabolizes Ap3A and Ap3N but not Ap4A or Ap4N in vivo. It is well known that Ap3A/Ap4A ratio is essential for functional activity of ApnA.© 2001 Academic Press

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361
Fhit is a homodimer as shown conclusively by crys-
tallographic analysis in two laboratories.8,9 Interest-
ingly, both in Drosophila and C. elegans the FHIT ho-
omologues are part of a larger fusion protein with ni-
trilase, i.e. NitFhit protein.10 Expression studies in
humans and mice have shown that this protein has
nearly identical expression patterns to Fhit, which,
together with the presence of the NitFhit fusion protein
in the worm and the fly, suggests their involvement in
the same biochemical pathway. Fhit protein expres-
sion is detected in epithelial cells in most human and
mouse tissues.3,11 The Fhit protein is mainly localized
in the cytoplasm and has been found in a complex
with tubulin and a ubiquitin conjugating enzyme.12,13

FHIT alterations in breast cancer

In tumours associated with environmental carcino-
gens, alterations in the FHIT gene occur early in
cancer development, but in other cancers this is
thought to be a late event and possibly associated
with cancer progression (reviewed by Croce et al., 1999).14
Cancer specific translocations have been mapped
within the FHIT gene in renal cell carcinoma
and a papilloma virus insertion site in cervical
carcinoma.15–17 The breakpoint at 3p14.2, involved
in the t(3;8) chromosome translocation of hereditary
renal cell carcinoma, interrupts the third intron of
the FHIT gene, inactivating one of the two FHIT
alleles.3 Frequent allelic losses at this region in
various malignancies, including breast carcinomas,
imply that FHIT may represent a TSG (Table 1). LOH
at the FHIT locus is detected in a subset of breast
tumours, with the highest frequency in familial breast
cancer lacking functional BRCA2 in the tumours,
presumably due to lack of DNA repair.19,21,23,24 Cell
lines from several tumour types, including breast
cancer, carry homozygous deletions at the FHIT locus
(Table 1).11,16,18,20 In addition to LOH, homozygous
deletions at chromosome 3p14 have been found in
sporadic breast cancer and benign proliferative
breast disease.27–29 No expression of Fhit was
observed, probably as a result of the homozygous
deletion.29 Additional reports suggest reduced
mRNA expression in a subset of breast cancer, and
FHIT gene loss has been shown to be associated with
reduced Fhit protein expression in both ductal and
lobular breast cancer (Table 2).19,22,25 Abnormal
sizes of mRNA have been reported to be tumour
specific in several cancer types, including breast
cancer (Table 2).19,20,30 The role of low abundance
aberrant FHIT transcripts is uncertain, particularly
when wild type transcripts are co-expressed in the
tumour cells. Still, a dominant negative mechanism
is possible. Hypermethylation in the FHIT promoter
region has been detected in 12/39 (31%) of primary
breast carcinomas and in 19/22 (86%) of breast
cancer cell lines.32 This methylation is allele specific
and results in reduced expression of the gene, as
detected by immunohistochemistry. Alterations at
the FHIT locus, or reduced expression of Fhit, has
been associated with breast tumour progression and
survival of breast cancer patients.26,31,35 In general,
the findings listed are in line with the tumour
suppressor function of the Fhit protein. However, it
has been argued that the FHIT gene may be altered
in cancers simply because it is located at a fragile
region and is likely to be susceptible to breakage. In
support of this view is the observation that somatic
point mutations in FHIT are rarely found in breast
or other tumours, and germline mutations do not
seem to be significant.20,34,35 As outlined in the next
section, the definite proof of a tumor suppressor
function of FHIT has been established by various
functional analyses.
Table 1. Deletions at the FHIT locus in primary breast tumours and homozygous deletions in breast cancer cell lines

<table>
<thead>
<tr>
<th>Deletions (LOHa):</th>
<th>Homozygous deletions:</th>
<th>Comment:</th>
<th>Reference:</th>
</tr>
</thead>
<tbody>
<tr>
<td>MB436b</td>
<td>Two deletions on each chromosome</td>
<td>11,16,18</td>
<td></td>
</tr>
<tr>
<td>8/32 25%</td>
<td>1/18 5%</td>
<td></td>
<td>19</td>
</tr>
<tr>
<td>9/22 41%</td>
<td>3/32 9%</td>
<td>LOH detected in premalignant tissue</td>
<td>20</td>
</tr>
<tr>
<td>20/45 44%</td>
<td>LOH detected in DCISc</td>
<td></td>
<td>21</td>
</tr>
<tr>
<td>8/49, 34/58 16%, 59%</td>
<td>Elevated LOH in ductal compared to lobular tumours</td>
<td></td>
<td>22</td>
</tr>
<tr>
<td>16/40, 12/19 40%, 63%</td>
<td>Elevated LOH in BRCA2 compared to sporadic tumours</td>
<td></td>
<td>23</td>
</tr>
<tr>
<td>33/150, 18/32 22%, 57%</td>
<td>Elevated LOH in BRCA2 compared to sporadic tumours</td>
<td></td>
<td>24</td>
</tr>
<tr>
<td>7/29, 22/29 24%, 76%</td>
<td>Elevated LOH in BRCA2 compared to sporadic tumours</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>76/239 32%</td>
<td>Association with patients’ survival</td>
<td></td>
<td>26</td>
</tr>
</tbody>
</table>

a Deletions are based on loss of heterozygosity results using microsatellite markers within the FHIT gene.
b The MB436 breast cancer cell line is well characterized with respect to FHIT deletions.
c Ductal carcinoma in situ.

Functional aspects of FHIT with respect to tumour growth

A variety of evidence argues for the tumour suppressor role of FHIT in breast cancer and other tumour types. Tumourigenicity can be reduced in lung, cervix and kidney cell lines after reintroduction of the FHIT gene.36–38 Of interest is the observation that a mutated form of Fhit, lacking the Ap3A hydrolase activity, also suppresses tumourigenicity, suggesting that the tumour suppressor activity of FHIT is not related to catalysis of nucleotide substrates.37 Perhaps the binding of Ap3A to Fhit is sufficient to suppress the malignant phenotype. Similarly, with the use of the human/mouse microcell hybrid, it was demonstrated that the introduction of chromosome 3 into a mouse fibrosarcoma line reduces tumourigenicity, whereas elimination of the FHIT locus enhances tumourigenesis.39 A definite proof of the tumour suppressor function of Fhit was demonstrated with the generation of Fhit knockout mice.40 Both heterozygous (Fhit+/−) and homozygous (Fhit−/−) Fhit knockout mice spontaneously develop tumours at increased frequency. Although some of the Fhit knockout mice have been reported to develop tumours with a similar spectrum to humans with Muir–Torre syndrome, the full spectrum of tumours that will develop spontaneously in Fhit+/− and Fhit−/− mice has not yet been reported.40,41 Both Fhit+/− and Fhit−/− mice are also more susceptible to carcinogen-induced tumours of the oesophagus and forestomach.40 Treatment of the Fhit knockout mice with adenovirus and AAV based expression of the Fhit reduces the malignant phenotype.41 Apart from the ability of Fhit to bind and hydrolyse Ap3A, little is known of the biochemical and biological function of the Fhit with respect to its tumour suppressor ability. Overexpression of Fhit in tumour cell lines lacking Fhit expression induces significant growth arrest, caused both by increased apoptosis and G1 cell cycle arrest.38,42,43 The ubiquitin conjugating enzyme, hUBC9, is associated with the C-terminal portion of Fhit, and this interaction is independent of Fhit enzymatic activity.13 Since the yeast UBC9 is involved in the degradation of S- and M-phase cyclins, Fhit may be involved in cell cycle control through its interaction with hUBC9.13 Similarly, it has been shown that intracellular concentration of ApnA can affect apoptotic pathways.44

FHIT loss: a marker of genetic instability or loss of a tumour suppression?

The FHIT gene is located at the most active common fragile site in the human genome, the aphidicolin sensitive site FRA3B.3 The fragility of this site can presumably be explained by late replication, which can be further delayed by inhibiting the DNA polymerase activity by aphidicolin.45 The behaviour of genomic fragile sites is not well documented in relation to tumour initiation and progression, but studies on the FHIT locus give an excellent opportunity to characterize such a site in cancer development. Tumour cells frequently exhibit genomic instability but little is known about the status...
Table 2. Expression of the FHIT gene in primary breast tumours

<table>
<thead>
<tr>
<th>Aberrant transcript:</th>
<th>Reduced mRNA expression a</th>
<th>Reduced protein expression b</th>
<th>Comment:</th>
<th>Reference:</th>
</tr>
</thead>
<tbody>
<tr>
<td>8/41</td>
<td>4/41</td>
<td>20%</td>
<td>Total abnormal expression in 30%</td>
<td>19</td>
</tr>
<tr>
<td>20%</td>
<td>10%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4/32</td>
<td>4/32</td>
<td>13%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23/61</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>38%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>46/77</td>
<td>Reduced expression</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60%</td>
<td>including DCIS c</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45/55</td>
<td>Reduced expression</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>82%</td>
<td>in lobular cancer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24/29</td>
<td>Low expression</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>83%</td>
<td>in BRCA2 tumours</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4/29</td>
<td>20/29</td>
<td>Association of abnormal transcript and absent Fhit protein</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>31%</td>
<td>69%</td>
<td>Association with hypermethylation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16/20</td>
<td>80%</td>
<td>Association with proliferation and tumour size</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>107/156</td>
<td>69%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>36/50</td>
<td>Association with tumour progression</td>
<td>33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>72%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Based on RT-PCR.
b Based on immunohistochemistry.
c Ductal carcinoma in situ.

do of common fragile sites in relation to this instability. Genetic instability in tumours can be divided into microsatellite instability (MIN) and chromosome instability (CIN) (reviewed by Cahill et al., 1999). The MIN phenotype is well characterized and is observed in almost all cases of HNPCC (hereditary non-polyposis colorectal carcinoma) and in a proportion of sporadic tumours of the colon, stomach, endometrium, lung, and some other tumour types. Intragenic microsatellite markers within introns of the FHIT gene show instability in MIN tumours to a similar level to microsatellites outside the gene. The relevance of these mutations to tumour development is, however, not clear. The MIN phenotype is rare in breast cancer. The CIN phenotype is not as well defined as the MIN phenotype, but a relatively high proportion (60–70%) of breast cancers show aneuploidy, that may reflect a CIN phenotype. LOH of FHIT intragenic markers have been found slightly elevated in aneuploid compared to diploid breast tumours (37% versus 28%), but this does not reach statistical significance. In the same study it was shown that LOH at the FHIT locus is associated with LOH at 12 different chromosome arms. One type of genomic instability that can be classified as CIN is detected in breast tumours from carriers of BRCA1 and BRCA2 germline mutations. Lack of proper DNA repair in these tumours is presumably the reason for this instability. These tumours show elevation of gains and losses of certain chromosomes that are characteristic, depending on whether the BRCA1 or BRCA2 function is lost. In BRCA2 mutated tumours, a frequent FHIT LOH is observed and the Fhit protein expression is reduced. As concluded from the analysis of LOH at several chromosomal regions, as distinct from 3p, in BRCA2 mutated tumours, the presence of a common fragile site alone cannot explain increased LOH frequency in these
tumours, and the growth advantage of the breast tumour cells may be of importance for the clonal selection of the chromosome losses. In general, the FHIT gene seems to be aberrant in tumours with at least certain types of genomic instability, and this suggests that it can serve as a marker for genomic instability. This unstable nature of FHIT in tumours does not, however, rule out its putative role as a tumour suppressor.

Conclusion

The protective effects of Fhit vis-à-vis tumorigenicity implies that Fhit is directly involved in the control of cell growth and/or proliferation. A definite mechanism to explain the anti-tumour role of Fhit has so far not been worked out. The acceptance of FHIT as a tumour suppressor has not been universal, since some reports suggest that the fragility of the locus alone could account for clonal or oligoclonal genetic alterations of FHIT in cancer. Still, compelling evidence on the effects of FHIT on cellular behaviour is in line with its role as a tumour suppressor. These data, together with frequent alterations of the FHIT locus in breast cancer, suggest its role in the pathogenesis of breast tumours.

References

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