seminars in **CANCER BIOLOGY**, Vol. 11, 2001: pp. 361–366
doi:10.1006/scbi.2001.0391, available online at http://www.idealibrary.com on IDEAL®

FHIT alterations in breast cancer

Sigurdur Ingvarsson

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

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).[1] Similarly, 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.[2] 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 promoter 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.[3] 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.[4] 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.[4,5] Murphy *et al.*[5] 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.[6]

From the Institute for Experimental Pathology, University of Iceland, Reykjavik, Iceland.

1044–579X/01/050361+ 06/$35.00/0

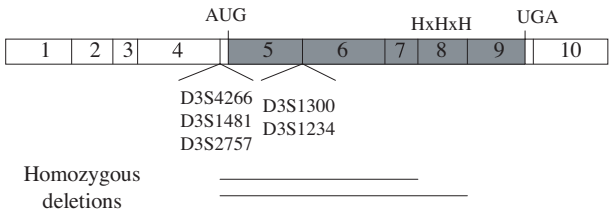

Figure 1. mRNA map of the FHIT together with information on the location of microsatellite markers frequently used for LOH studies in breast cancer. Lines represent frequent homozygous deletions detected in breast cancer cell lines. The H × H × H represents the histidine triad at the catalytic site of the protein. The shaded exons 5–9 are coding, while exons 1–4 and 10 are non-coding.

In addition to Fhit the Ap4A/Ap3A ratio is controlled by other proteins. TrpRS specifically stimulates the production of Ap3A while Fhit degrades it and the Ap4A/Ap3A ratio can therefore be modulated by changing the activity/ratio of TrpRS/Fhit proteins.[7] The function of ApnA has been associated with several cell growth control functions, such as the stimulation of DNA synthesis, mitogen activity, gene transcription and the regulation of membrane bound ion channels.[6]

Fhit is a homodimer as shown conclusively by crystallographic analysis in two laboratories.[8,9] Interestingly, both in *Drosophila* and *C. elegans* the FHIT homologues are part of a larger fusion protein with nitrilase, 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 expression 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 carcinogens, 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.[26,31,33] 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

Deletions (LOH[a]):	Homozygous deletions:	Comment:	Reference:
	MB436[b]	Two deletions on each chromosome	11,16,18
8/32 25%	1/18 5%		19
9/22 41%	3/32 9%	LOH detected in premalignant tissue	20
20/45 44%		LOH detected in DCIS [c]	21
8/49, 34/58 16%, 59%		Elevated LOH in ductal compared to lobular tumours	22
16/40, 12/19 40%, 63%		Elevated LOH in BRCA2 compared to sporadic tumours	23
33/150, 18/32 22%, 57%		Elevated LOH in BRCA2 compared to sporadic tumours	24
7/29, 22/29 24%, 76%		Elevated LOH in BRCA2 compared to sporadic tumours	25
76/239 32%		Association with patients' survival	26

[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

Aberrant transcript:	Reduced mRNA expression[a]:	Reduced protein expression[b]:	Comment:	Reference:
8/41 20%	4/41 10%		Total abnormal expression in 30%	19
4/32 13%				20
23/61 38%				30
		46/77 60%	Reduced expression, including DCIS[c]	25
		45/55 82%	Reduced expression in lobular cancer	22
		24/29 83%	Low expression in BRCA2 tumours	25
9/29 31%		20/29 69%	Association of abnormal transcript and absent Fhit protein	31
		16/20 80%	Association with hypermethylation	32
		107/156 69%	Association with proliferation and tumour size	31
		36/50 72%	Association with tumour progression	33

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

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).[46] 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.[47] 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.[48–50] 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.[26] In the same study it was shown that LOH at the FHIT locus is associated with LOH at 12 different chromosome arms.[26] One type of genomic instability that can be classified as CIN is detected in breast tumours from carriers of BRCA1 and BRCA2 germline mutations.[24,51] 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.[24,25] 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.[24,52] 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

1. Ingvarsson S (1999) Molecular genetics of breast cancer progression. Semin Cancer Biol 9:277–288
2. Berx G, Cleton-Jansen AM, Strumane K, Leeuw WJF, Nollet F, Roy F, Cornelisse C (1996) E-cadherin is inactivated in a majority of invasive lobular breast cancers by truncation mutations throughout its extracellular domain. Oncogene 13:1919–1925
3. Ohta M *et al.* (1996) The FHIT gene, spanning the chromosome 3p14.2 fragile site and renal carcinoma-associated t(3;8) breakpoint, is abnormal in digestive tract cancers. Cell 84:587–597
4. Barnes LD, Garrison PN, Siprashvili Z, Guranowski A, Robinson AK, Ingram SW, Croce CM, Ohta M, Huebner K (1996) Fhit, a putative tumor suppressor in humans, is a dinucleoside 5′, 5‴-P1, P3- triphosphate hydrolase. Biochemistry 35:11529–11535
5. Murphy GA, Halliday D, McLennan AG (2000) The Fhit tumor suppressor protein regulates the intracellular concentration of diadenosine triphosphate but not diadenosine tetraphosphate. Cancer Res 60:2342–2344
6. Kissilev LL, Justesen J, Wolfson AD, Frolova LY (1998) Diadenosine oligophosphates (ApnA), a novel class of signalling molecules? FEBS Lett 427:157–163
7. Kissilev LL, Wolfson AD (1994) Aminoacyl-transfer-RNA synthetases from higher eukaryotes. Prog Nucleic Acid Res Mol Biol 48:83–142
8. Lima CD, DAmico KL, Naday I, Rosenbaum G, Westbrook EM, Hendrickson WA (1997) MAD analysis of FHIT, a putative human tumor suppressor from the HIT protein family. Structure 5:763–774
9. Pace HC *et al.* (1998) Genetic, biochemical, and crystallographic characterization of Fhit-substrate complexes as the active signaling form of Fhit. Proc Natl Acad Sci USA 95:5484–5489
10. Pekarsky Y *et al.* (1998) Nitrilase and Fhit homologs are encoded as fusion proteins in Drosophila melanogaster and Caenorhabditis elegans. Proc Natl Acad Sci USA 95:8744–8749
11. Druck T *et al.* (1997) Structure and expression of the human FHIT gene in normal and tumor cells. Cancer Res 57:504–512
12. Chaudhuri AR, Khan IA, Prasad V, Robinson AK, Luduena RF, Barnes LD (1999) The tumor suppressor protein Fhit—a novel interaction with tubulin. J Biol Chem 274:24378–24382
13. Shi YF, Zou MJ, Farid NR, Paterson MC (2000) Association of FHIT (fragile histidine triad), a candidate tumour suppressor gene, with the ubiquitin-conjugating enzyme hUBC9. Biochem J 352:443–448
14. Croce CM, Sozzi G, Huebner K (1999) Role of FHIT in human cancer. J Clin Oncol 17:1618–1624
15. Gemmill RM, West JD, Boldog F, Tanaka N, Robinson LJ, Smith DI, Li F, Drabkin HA (1998) The hereditary renal cell carcinoma 3;8 translocation fuses FHIT to a patched- related gene, TRC8. Proc Natl Acad Sci USA 95:9572–9577
16. Inoue H, Ishii H, Alder H, Snyder E, Druck T, Huebner K, Croce CM (1997) Sequence of the FRA3B common fragile region: implications for the mechanism of FHIT deletion. Proc Natl Acad Sci USA 94:14585–14589
17. Wilke CM, Hall BK, Hoge A, Paradee W, Smith DI, Glover TW (1996) FRA3B extends over a broad region and contains a spontaneous HPV16 integration site: direct evidence for the coincidence of viral integration sites and fragile sites. Hum Mol Genet 5:187–195
18. Kastury K *et al.* (1996) Potential gastrointestinal tumor suppressor locus at the 3p14.2 FRA3B site identified by homozygous deletions in tumor cell lines. Cancer Res 56:978–983
19. Negrini M, Monaco C, Vorechovsky I, Ohta M, Druck T, Baffa R, Huebner K, Croce CM (1996) The FHIT gene at 3p14.2 is abnormal in breast carcinomas. Cancer Res 56:3173–3179
20. Ahmadian M *et al.* (1997) Analysis of the FHIT gene and FRA3B region in sporadic breast cancer, preneoplastic lesions, and familial breast cancer probands. Cancer Res 57:3664–3668
21. Man S, Ellis I, Sibbering R, Blamey RW, Brook JD (1996) High levels of allele loss at FHIT and ATM genes in non-comedo ductal carcinoma *in situ* and grade I tubular invasive breast cancers. Cancer Res 56:5484–5489
22. Huiping C, Jonasson JG, Agnarsson BA, Sigbjornsdottir BI, Huebner K, Ingvarsson S (2000) Analysis of the fragile histidine triad (FHIT) gene in lobular breast cancer. Eur J Cancer 36:1552–1557
23. Bergthorsson JT, Johannsdottir JT, Jonasdottir A, Eiriksdottir G, Egilsson V, Ingvarsson S, Barkardottir RB, Arason A (1998) Chromosome imbalance at the 3p14 region in human breast tumours: high frequency in patients with inherited predisposition due to BRCA2. Eur J Cancer 34:142–147
24. Ingvarsson S *et al.* (1998) High incidence of loss of heterozygosity in breast tumours from carriers of the BRCA2

999del5 mutation. Cancer Res 58:4421–4425
25. Ingvarsson S *et al.* (1999) Reduced Fhit expression in sporadic and BRCA2-linked breast carcinomas. Cancer Res 59:2682–2689
26. Ingvarsson S, Sigbjornsdottir BI, Huiping C, Jonasson JG, Agnarsson BA (2001) Alterations of the FHIT gene in breast cancer: association with tumour progression and patient survival. Cancer Detect Prev 25:292–298
27. Buchhagen DL, Qiu L, Etking P (1994) Homozygous deletions, rearrangements and hypermethylation implicate chromosome 3p14.3—3p21.3 in sporadic breast cancer development. Int J Cancer 57:473–479
28. Chen LC, Matsumura K, Deng G, Kurisu W, Ljung BM, Lerman MI, Waldman FM, Smith H (1994) Deletion of two separate regions on chromosome 3p in breast cancer. Cancer Res 54:3021–3024
29. Panagopoulos I, Pandis N, Thelin S, Petersson C, Mertens F, Borg A, Kristoffersson U, Mitelman F, Aman P (1996) The FHIT and PTPRG genes are deleted in benign proliferative breast disease associated with familial breast cancer and cytogenetic rearrangements of chromosome band 3p14.2. Cancer Res 56:4871–4875
30. Hayashi S, Tanimoto K, Hajiro-Nakanishi K, Tsuchiya E, Kurosumi M, Higashi Y, Imai K, Suga K, Nakachi K (1997) Abnormal FHIT transcripts in human breast carcinomas: a clinicopathological and epidemiological analysis of 61 Japanese cases. Cancer Res 57:1981–1985
31. Campliglio M, Pekarsky Y, Menard S, Tagliabue E, Pilotti S, Croce CM (1999) FHIT loss of function in human primary breast cancer correlates with advanced stage of disease. Cancer Res 59:3866–3869
32. Zochbauer-Muller S, Fong KM, Maitra A, Lam S, Geradts J, Ashfaq R, Virmani AK, Milchgrub S, Gazdar AF, Minna JD (2001) 5'CpG island methylation of the FHIT gene is correlated with loss of gene expression in lung and breast cancer. Cancer Res 61:3581–3585
33. Gatalica Z, Lele S, Rampy BA, Norris BA (2000) The expression of Fhit protein is related inversely to disease progression in patients with breast carcinoma. Cancer 88:1378–1383
34. Gemma A, Hagiwara K, Ke Y, Burke LM, Khan MA, Nagashima M, Bennet WP, Harris CC (1997) FHIT mutations in human primary gastric cancer. Cancer Res 57:1435–1437
35. Kannan K, Munirajan AK, Bhuvarahamurthy V, Mohanprasad BKC, Shankar P, Tsuchida N, Shanmugam G (2000) FHIT gene mutations and single nucleotide polymorphism in Indian oral and cervical squamous cell carcinomas. Oral Oncol 36:189–193
36. Ji L, Fang B, Yen N, Fong K, Minna JD, Roth JA (1999) Induction of apoptosis and inhibition of tumorigenicity and tumor growth by adenovirus vector-mediated fragile histidine triad (FHIT) gene overexpression. Cancer Res 59:3333–3339
37. Siprashvili Z *et al.* (1997) Replacement of Fhit in cancer cells suppresses tumorigenicity. Proc Natl Acad Sci USA 94:13771–13776
38. Werner NS, Siprashvili Z, Fong LY, Marquitan G, Schroder JK, Bardenheuer W, Seeber S, Huebner K, Schutte J, Opalka B (2000) Differential susceptibility of renal carcinoma cell lines to tumor suppression by exogenous Fhit expression. Cancer Res 60:2780–2785
39. Kholodnyuk ID, Szeles A, Yang Y, Klein G, Imreh S (2000) Inactivation of the human fragile histidine triad gene at 3p14.2 in monochromosomal human/mouse microcell hybrid-derived severe combined immunodeficient mouse tumors. Cancer Res 60:7119–7125
40. Fong LY *et al.* (2000) Muir–Torre-like syndrome in Fhit-deficient mice. Proc Natl Acad Sci USA 97:4742–4747
41. Dumon KR *et al.* (2001) FHIT gene therapy prevents tumor development in Fhit-deficient mice. Proc Natl Acad Sci USA 98:3346–3351
42. Guo ZY, Vishwanatha JK (2000) Effect of regulated expression of the fragile histidine triad gene on cell cycle and proliferation. Mol Cell Biochem 204:83–88
43. Sard L *et al.* (1999) The tumor-suppressor gene FHIT is involved in the regulation of apoptosis and in cell cycle control. Proc Natl Acad Sci USA 96:8489–8492
44. Vartanian A, Prudovsky I, Suzuki H, DalPra I, Kisselev L (1997) Opposite effects of cell differentiation and apoptosis on Ap(3)A/Ap(4)A ratio in human cell cultures. FEBS Lett 415:160–162
45. Wang L, Darling J, Zhang JS, Huang H, Liu W, Smith DI (1999) Allele-specific late replication and fragility of the most active common fragile site, FRA3B. Hum Molec Genet 8:431–437
46. Cahill DP, Kinzler KW, Vogelstein B, Lengauer C (1999) Genetic instability and darwinian selection in tumours. Trends Cell Biol 9:M57–M60
47. Huiping C, Johannsdottir JT, Arason A, Olafsdottir GH, Eiriksdottir G, Egilsson V, Ingvarsson S (1999) Replication error in human breast cancer: comparison with clinical variables and family history of cancer. Oncol Rep 6:117–122
48. Beerman H, Kluin PM, Hermans J, Van de Velde CJ, Cornelisse CJ (1990) Prognostic significance of DNA-ploidy in a series of 690 primary breast cancer patients. Int J Cancer 45:34–39
49. Hedley DW, Rugg CA, Gelber RD (1987) Association of DNA index and S-phase fraction with prognosis of nodes positive early breast cancer. Cancer Res 47:361–366
50. Toikkanen S, Joensuu H, Klemi P (1989) The prognostic significance of nuclear DNA content in invasive breast cancer—a study with long-term follow-up. Br J Cancer 60:693–700
51. Tirkkonen M *et al.* (1997) Distinct somatic genetic changes associated with tumor progression in carriers of BRCA1 and BRCA2 germ-line mutations. Cancer Res 57: 1222–1227
52. Sigbjornsdottir BI, Ragnarsson G, Agnarsson BA, Huiping C, Barkardottir RB, Egilsson V, Ingvarsson S (2000) Chromosome 8p alterations in sporadic and BRCA2 999del5 linked breast cancer. J Med Genet 37:342–347