Development of spontaneous tumours and intestinal lesions in \textit{Fhit} gene knockout mice

The fragile histidine triad (\textit{Fhit}) gene has been identified as a candidate tumour suppressor gene localized in \textit{FRA3B}, the most sensitive common fragile site, at chromosome 3p14.2 (Ohta \textit{et al.}, 1996). Chromosomal deletion of the \textit{Fhit}-containing locus or inactivation of \textit{Fhit} is frequently observed in various types of cancers (for a review, see Huebner and Croce, 2003), consistent with a tumour suppressor function in a variety of organs. The \textit{Fhit} protein carries a proapoptotic activity through a caspase-dependent pathway in human cancer cells, which may contribute to the tumour suppressor activity (Ji \textit{et al.}, 1999; Ishii \textit{et al.}, 2001; Roz \textit{et al.}, 2002).

Previously, we have demonstrated that \textit{Fhit} mutant mice develop tumours spontaneously in lymphatic tissues, sebaceous glands, liver, stomach, colonic submucosa, uterus, skin, salivary glands, and parathyroid glands (Zanesi \textit{et al.}, 2001). Moreover, \textit{Fhit} knockout mice are susceptible to chemical carcinogen-induced tumour formation in the forestomach (Fong \textit{et al.}, 2000), which is reversed by adenoviral transduction of the human \textit{Fhit} gene (Dumon \textit{et al.}, 2001). These results are consistent with the tumour suppressor function of \textit{Fhit}. To further characterise tissue types affected by inactivation of \textit{Fhit}, including nontumorous lesions, we have constructed a second mouse strain with a targeted \textit{Fhit} gene, where germline and somatic mutations were detected. Amplified DNA samples were sequenced with anti-\textit{\beta-catenin} antibody (Sigma Chemical Co., St Louis, MO, USA) at 5000-fold dilution or with c-Kit antibody (Santa Cruz, CA, USA) at 400-fold. Signals were visualised using the Vectastain Elite Kit (Vector Laboratories, Burlingame, CA, USA). For mutation analysis and microsatellite instability (MSI) analysis, DNA samples were extracted from paraffin-embedded sections using DEXPAT (TaKaRa, Japan). Extracted DNA samples were subjected to PCR in 11 contiguous fragments spanning nucleotides 2750–4830 of exon 15 of the \textit{Apc} gene, where germline and somatic mutations were frequently detected. Amplified DNA samples were sequenced directly with the respective primers using the BigDye Terminator.
Cycle Sequencing Ready Reaction Kit (Applied Biosystems; Rotkreuz, Switzerland) and ABI Prism 377 DNA Sequencing System (Applied Biosystems). For MSI analysis, four primer sets, D1Mit4, D6Mit59, D9Mit67 and D10Mit2 (http://www.informatics.jax.org), were used. DNA samples extracted from polyps were amplified by PCR. After denaturation (100°C, 5 min), PCR products were loaded onto 5% Long Ranger Gel (TaKaRa) in 8 M urea, and electrophoresed at 150 V for 40 min. The dried gel was scanned in a BAS-1800 (Fujifilm, Japan).

RESULTS AND DISCUSSION

Fhit (+/–) and (–/–) mice were viable, fertile and clinically normal up to 12 months of age. However, upon necropsy, tumours and abnormal lesions were found in mice older than 12 months, consistent with observations of another Fhit mutant strain (Fong et al, 2000). Although several types of tumours developed spontaneously even in the age-matched wild-type littermates, overall incidences of tumours and abnormal lesions were significantly higher in both Fhit (+/–) and (–/–) mice than in Wnt pathway was activated in the intestinal polyps of Fhit-deficient mice, we localised Fhit/b-catenin had accumulated in the nucleus in two polyps of Fhit (+/–) mice because of Fhit haploinsufficiency, either directly or through an indirect mechanism.

Mutations in the gene encoding Apc or b-catenin result in intestinal adenomatous polyposis through Wnt signalling activation (Oshima et al, 1995; Harada et al, 1999). To examine whether the Wnt pathway was activated in the intestinal polyps of Fhit-deficient mice, we localised b-catenin in nine polyp tissues from both Fhit (+/–) and Fhit (–/–) mice by immunohistochemistry. b-Catenin had accumulated in the nucleus in two polyps of Fhit (+/–) mice, indicating Wnt activation in these adenoma cells (Figure 1E). b-Catenin was localised to the basolateral side of adenoma cells in the other seven polyps (Figure 1F). By sequence
Tumours and intestinal lesions in Fhit mutants

T Fujishita et al

British Journal of Cancer (2004) 91(8), 1571 – 1574

© 2004 Cancer Research UK

Genetics and Genomics
analysis of the \textit{Apc} gene of the two nuclear $\beta$-catenin-positive polyps, we further found a nonsense mutation in the \textit{Apc} gene at codon 1055 (wt: GAA (Glu) $\rightarrow$ mutant: TAA (STOP)) in one polyp. This mutation may explain the cause of at least a fraction of the polyps, although other genes may be mutated giving rise to other adenomas. As the intestines were not inspected in detail in the other \textit{Fhit} knockout strain, it is conceivable that similar intestinal lesions existed at low frequencies. These results suggest that an insufficient \textit{Fhit} level can induce \textit{Apc} gene mutations, which is consistent with the enhanced survival and mutation frequency of \textit{Fhit}-deficient cells after UVC or mitomycin C damage (Ottey \textit{et al}, 2004). In contrast, \textit{MSI} was not observed in genomic DNAs from \textit{Fhit} mutant mouse tails or tumours (Fong \textit{et al}, 2000). In the present study, we also examined \textit{MSI} in cells from two intestinal polyps, using four different sets of markers, and confirmed no \textit{MSI} in these samples (data not shown). Accordingly, it is possible that inactivation of \textit{Fhit} results in tumorigenesis through induction of mutations in tumour suppressor genes.

**REFERENCES**

Dumon KR, Ishii H, Fong LY, Zanesi N, Fidanza V, Mancini R, Vecchione A, Baffa R, Trapasso F, During MJ, Huebner K, Croce CM (2001) \textit{Fhit} gene therapy prevents tumor development in \textit{Fhit}-deficient mice. \textit{Proc Natl Acad Sci USA} 98: 3346–3351

Fong LY, Fidanza V, Zanesi N, Lock LF, Siracusa LD, Mancini R, Siprashvili Z, Ottey M, Martin SE, Druck T, McCue PA, Croce CM, Huebner K (2000) Muir–Torre-like syndrome in \textit{Fhit}-deficient mice. \textit{Proc Natl Acad Sci USA} 97: 4742–4747

Harada N, Tamai Y, Ishikawa T, Sauer B, Takaku K, Oshima M, Taketo MM (1999) Intestinal polyposis in mice with a dominant stable mutation of the $\beta$-catenin gene. \textit{EMBO J} 18: 5931–5942

Huebner K, Croce CM (2003) Cancer and the \textit{FRA3B/FHIT} fragile locus: it’s a HIT. \textit{Br J Cancer} 88: 1501–1506

Ishii H, Dumon KR, Vecchione A, Trapasso F, Mimori K, Alder H, Mori M, Sozzi G, Baffa R, Huebner K, Croce CM (2001) Effect of adeno viral transduction of the fragile histidine triad gene into esophageal cancer cells. \textit{Cancer Res} 61: 1578–1584

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 (\textit{Fhit}) gene overexpression. \textit{Cancer Res} 59: 3333–3339

Ohta M, Inoue H, Cotticelli MG, Kastury K, Baffa R, Palazzo J, Siprashvili Z, Mori M, McCue P, Druck T, Croce CM, Huebner K (1996) The \textit{Fhit} gene, spanning the chromosome 3p14.2 fragile site and renal carcinoma- associated 3p6.3 breakpoint, is abnormal in digestive tract cancers. \textit{Cell} 84: 587–597

Oshima M, Sugiyama H, Kitagawa K, Kobayashi M, Itakura C, Taketo M (1995) Loss of \textit{Apc} heterozygosity and abnormal tissue building in nascent intestinal polypsis in mice carrying a truncated \textit{Apc} gene. \textit{Proc Natl Acad Sci USA} 92: 4842–4846

Ottey M, Han S-Y, Druck T, Barnoski B, McCorkell KA, Croce CM, Raventos-Suarez C, Fairchild CR, Wang Y, Huebner K (2004) \textit{Fhit} deficient normal and cancer cells are mitomycin C and UVC resistant. \textit{Br J Cancer} (in press)

Roz L, Gramegna M, Ishii H, Croce CM, Sozzi G (2002) Restoration of fragile histidine triad (\textit{Fhit}) expression induces apoptosis and suppresses tumorigenicity in lung and cervical cancer cell lines. \textit{Proc Natl Acad Sci USA} 99: 3615–3620

Workman P, Twentyman P, Balkwill F, Balmain A, Chaplin D, Double J, Embleton J, Newell D, Raymond R, Stables J, Stephens T, Wallace J (1998) United Kingdom Co-ordinating Committee on Cancer Research (UKCCCR) Guidelines for the Welfare of Animals in Experimental Neoplasia (Second Edition). \textit{Br J Cancer} 77: 1–10

Zanesi N, Fidanza V, Fong LY, Mancini R, Druck T, Valtieri M, Rudiger T, McCue PA, Croce CM, Huebner K (2001) The tumor spectrum in \textit{Fhit}-deficient mice. \textit{Proc Natl Acad Sci USA} 98: 10250–10255

On the other hand, morphological abnormalities in the small intestine, such as swollen crypts (goblet cell hyperplasia, Figure 1G, H) and fused villi (aggregated villi, Figure 1I, J), were observed in both \textit{Fhit} (+/−) and (−/−) mice at similar incidences. Such lesions were not found in the age-matched wild-type mice. Histologically, these lesions consisted of differentiated epithelial cells without any signs of dysplasia. Thus, it is conceivable that \textit{Fhit} expression is necessary also for the maintenance of normal intestinal architecture, in addition to suppressing tumorigenesis.

**ACKNOWLEDGEMENTS**

We thank Dr M Ohta for discussion of the \textit{Fhit} gene targeting construct and Y Toda for technical advice on immunostaining. This work was supported by grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan.