

Original Article

The effects of Fhit on tumorigenesis after multi-exposure to low-dose radiation

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Abstract: Low-dose (≤ 0.1 Gy) radiation could reduce high-dose induced damage including tumorigenesis. However, it remains unclear whether multi-exposure to low-dose radiation at a high dose rate has any risk for increasing tumorigenesis, and whether Fhit plays any role in the process. The purpose of this study is to investigate the effects of multi-exposure to low-dose radiation at a high dose rate on tumorigenesis, and the role of Fhit in it. We irradiated Fhit^{+/+} and Fhit^{-/-} mice with 1 Gy/1 or 0.1 Gy x 10 exposures at a dose rate of 1 Gy/min, sacrificed the mice at 1.5 years after radiation and observed multi-organ tumorigenesis. The results showed that although the spontaneous tumorigenesis in these mice was relatively high, 1 Gy/1-exposure dramatically increased the tumorigenesis including lung and liver tumor. Fhit^{-/-} mice showed more tumorigenesis than Fhit^{+/+} mice after 1 Gy/1-exposure. However, 0.1 Gy x 10 exposures did not increase tumorigenesis, and there was no statistical difference in tumorigenesis between Fhit^{+/+} mice and Fhit^{-/-} mice following 0.1 Gy x 10 exposures. Our results suggest that 0.1 Gy, even after multiple exposures, does not increase tumorigenesis, and Fhit could prevent high-dose radiation-induced tumors but has no effect in a low-dose environment.

Key words: Fhit, low dose, ionizing radiation, tumorigenesis

Introduction

It has been known for decades that ionizing radiation increases carcinogenesis frequency. Recently, more and more data indicate that low-dose radiation (≤ 0.1 Gy) might not increase the risk for carcinogenesis. Low doses of ionizing radiation (IR) at ≤ 0.1 Gy exposure could induce cell resistance to the high-dose IR-induced damage [1]. The adaptive response was then found to reduce gene mutation rates in irradiated cells [2-10] and prevented the mice from the following high dose IR-induced tumors [11, 12]. These results suggest that low dose (≤ 0.1 Gy) radiation might not be harmful to the human body. However, it remains unclear whether multi-exposure to low dose radiation (≤ 0.1 Gy) at a high dose rate could promote carcinogenesis and whether Fhit plays any role in the process.

Fhit is a gene related to human tumor progression [13], and plays a protective role in preventing mice from carcinogen induced tumors [14]. We previously reported that Fhit could prevent human cells from high-doses of UV and ionizing radiation-induced *HPRT* mutation [15, 16], suggesting that Fhit prevents DNA damage-induced carcinogenesis. However, it remains unclear whether Fhit could prevent high-dose radiation induced carcinogenesis and whether it plays any role in a low-dose environment. To examine whether multi-exposure of low dose radiation (≤ 0.1 Gy) at a high dose rate promotes carcinogenesis and whether Fhit plays a protective role in the process, we designed this study. The results suggest that 0.1 Gy at 1 Gy/min, even multiple exposures, does not increase tumorigenesis and Fhit could prevent high-dose radiation-induced tumors but has no effect in a low-dose environment.

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Materials and methods

Mice

C57B16/J strain, wild type (Fhit^{+/+}) and Fhit knockout (Fhit^{-/-}) mice, 4-6 weeks old, 240 in 6 groups were used in this experiment (**Table 1**). The mice were sacrificed at 1.5 years after exposure to ionizing radiation. The multi-organs including lungs, liver, bones, kidneys, intestines and multi-glands were taken for histological slide preparation. The samples were fixed in buffered formalin and sent to the University's pathology facility for tissue slicing, and hematoxylin & eosin (H&E) staining. Because most tumors observed in different or-

Differences of tumorigenesis between the treatment groups were calculated by chi-square test. Grading data were calculated by riddit analysis. *P* values < 0.05 were regarded as significant.

Results

Whole body exposure to 0.1 Gy X 10 did not increase the frequencies of multi-organ tumorigenesis

To investigate the effects of multi-exposure to low dose (≤ 0.1 Gy) radiation on tumorigenesis, we compared tumorigenesis among six groups of C57B16/J strain mouse (Fhit^{+/+} and

Table 1. Mice groups

Radiation	Fhit ^{+/+}		Fhit ^{-/-}	
	Male	Female	Male	Female
0 Gy	20	20	20	20
0.1 Gy x10	20	20	20	20
1 Gy	20	20	20	20

Table 2. Total Tumorigenesis Frequency

Group	Radiation	Tumorigenesis Frequency
Fhit ^{+/+}	0 Gy	(12/40) 30%
Fhit ^{+/+}	0.1 Gy x 10	(14/40) 35%
Fhit ^{+/+}	1 Gy	(24/40) 60%*
Fhit ^{-/-}	0 Gy	(14/40) 35%
Fhit ^{-/-}	0.1 Gy x 10	(12/40) 30%
Fhit ^{-/-}	1 Gy	(30/40) 75%**

p<0.05, ** *p*<0.01 (Comparison between irradiated group and non-irradiated group)

gans were as nests and invisible by eye directly, the size and frequency of the tumors were observed with a microscope (Nikon, Tokyo, Japan). The number of mice that generated tumors, tumor number and the tumor size in the liver or lung organ per group were analyzed and scored by using Zeiss software.

Irradiation

The mice (whole body) were exposed to cesium source (γ -ray) and irradiated with 1 Gy either one exposure or fractionated into 10 exposures (0.1 Gy x 10), in 10 days at the dose rate of 1 Gy/min.

Statistical analysis

Fhit^{-/-} as indicated in **table 1**) including: the mice without radiation, the mice exposed to 0.1 Gy x 10 exposures (0.1 Gy/exposure, 1 exposure/day), and the mice exposed to 1 Gy (1 exposure). We sacrificed the mice at 1.5 years following radiation and observed the number of mice with tumors, tumor number and tumor size in multi-organs including lungs, liver, bones, kidneys, intestines, ovaries, testis and salivary glands. The results showed that although the Fhit^{+/+} mice had a high spontaneous carcinogenesis in multi-organs, especially in the liver and lung (total tumorigenesis frequency ~30% and some mice showed multi-organ tumors) (**Figure 1**) (**Table 2**), Fhit^{-/-} mice didn't show increased tumorigenesis without radiation. Multi-exposure of 0.1 Gy (0.1 Gy x

Tumor in different organs (x100)

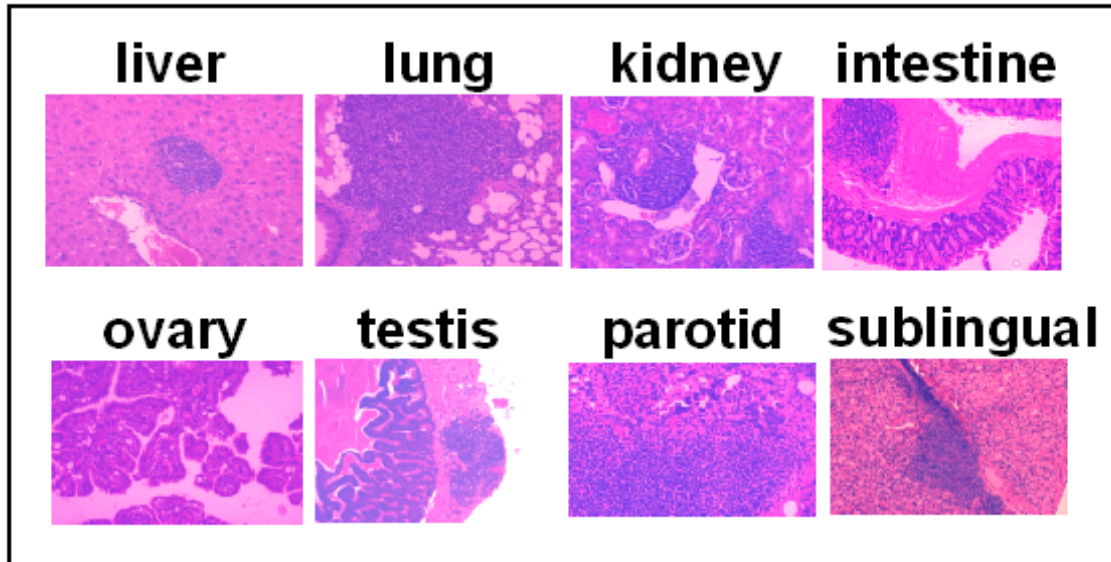


Figure 1. Different type tumors were observed in the mice. The mice were sacrificed as described in Materials and Methods. The multi-organs including lung, liver, kidney, intestine and multi-glands including ovary, testis, parotid and sublingual gland were taken for histological slide preparation. The samples were fixed in buffered formalin and sent to the University' pathology facility for tissue slicing and hematoxylin & eosin (H&E) staining. The magnification is 100 X.

Table 3. Liver Tumor Index

Group	Radiation	+	++	+++	++++	Index
Fhit ^{+/+}	0 Gy	7	2	1	0	14
Fhit ^{+/+}	0.1 Gy x 10	9	1	1	0	14
Fhit ^{+/+}	1 Gy	15	3	1	1	<u>28**</u>
Fhit ^{-/-}	0 Gy	7	2	1	0	14
Fhit ^{-/-}	0.1 Gy x 10	7	2	0	0	11
Fhit ^{-/-}	1 Gy	11	8	5	4	<u>58**/*</u>

* $p < 0.05$ ** $p < 0.01$ (Comparison between irradiated group and non-irradiated group, /*: Comparison between 1 Gy-irradiated Fhit^{+/+} and Fhit^{-/-} groups)

Table 4. Lung Tumor Index

Group	Radiation	+	++	+++	++++	Index
Fhit ^{+/+}	0 Gy	10	1	0	0	12
Fhit ^{+/+}	0.1 Gy x 10	9	1	0	0	11
Fhit ^{+/+}	1 Gy	17	5	0	0	<u>27**</u>
Fhit ^{-/-}	0 Gy	6	3	1	0	15
Fhit ^{-/-}	0.1 Gy x 10	8	3	0	0	14
Fhit ^{-/-}	1 Gy	10	6	6	4	<u>56**/*</u>

* $p < 0.05$ ** $p < 0.01$ (Comparison between irradiated group and non-irradiated group, /*: Comparison between 1 Gy-irradiated Fhit^{+/+} and Fhit^{-/-} groups)

10) did not increase the tumorigenesis frequency in Fhit^{+/+} or Fhit^{-/-} mice. Sex glands including ovary and testis tumorigenesis also

did not increase frequencies in 0.1 Gy x 10 irradiated mice and there was no difference in the sex gland tumorigenesis between Fhit^{+/+}

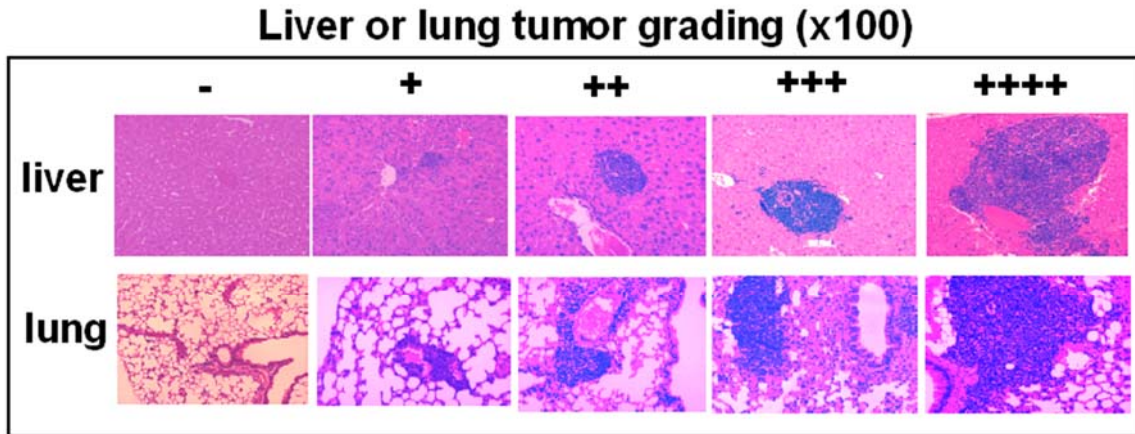


Figure 2. Grade of the size in liver or lung tumors. The size of liver or lung tumors was graded as: - (no tumor), + (tumor size < 0.1 mm), ++ (tumor size is between 0.1-0.5 mm), +++ (tumor size is between 0.5-1mm), and +++++ (tumor size > 1 mm). The indexes were calculated by combining the diameters of all liver or lung tumors in each mouse. The magnification is 100 X.

and Fhit^{-/-} mice after exposure to 0.1 Gy x 10. However, 1 Gy with 1 exposure dramatically increased the tumorigenesis frequency in Fhit^{+/+} and Fhit^{-/-} mice when compared with that in non-irradiated control mice (P<0.01) (Table 2). These results suggest that ≤0.1 Gy is a safe dose for tumorigenesis even at a high dose rate with multiple exposures. Although tumorigenesis frequency is higher in Fhit^{-/-} mice (75%) than in Fhit^{+/+} mice (60%) following 1 Gy/1 exposure, there is no statistical difference between these two groups, which might be due to the small number of animals in each group (40 mice). We then did the following analysis.

Fhit prevents high-dose radiation promoted tumorigenesis

To further investigate the effects of Fhit on high-dose radiation promoted tumorigenesis, we analyzed the tumor size in the lung and liver organs from Fhit^{+/+} and Fhit^{-/-} mice following radiation (0.1 Gy x 10 or 1 Gy x 1). We divided the tumor size into four grades by observing the sections under microscopy: +: tumor diameter < 0.1 mm; ++: tumor diameter 0.1-0.5 mm; +++: tumor diameter 0.5-1 mm; +++++: tumor diameter > 1 mm (Figure 2). We calculated the tumor diameter by combining tumors in one mouse if the mouse had more tumors in liver or lung. We scored the total tumor index by using the number of mice bearing tumors in each category to time grade: for example, 2 mice with tumor + equaling tumor

index 2 (derived from 2 x 1) and 2 mice with tumor ++ equaling tumor index 4 (derived from 2 x 2). In this way, we compared the index for liver and lung tumors among different groups. The results showed that both 1 Gy/1 exposure irradiated Fhit^{+/+} and Fhit^{-/-} mice dramatically increased both indexes of liver and lung tumors when compared with non-irradiated control groups and 0.1 Gy x 10 exposures irradiated groups (Table 3 and 4) (P< 0.01). Interestingly, although there is no statistical difference of total liver or lung tumor frequency between Fhit^{+/+} and Fhit^{-/-} mice following 1 Gy/1 exposure, there are significant differences in the indexes for both liver and lung tumors between the two groups: Fhit^{+/+} group showed lower indexes of liver and lung tumors than in Fhit^{-/-} group (Table 3 and 4) (P< 0.05). These results further confirm that low-dose (0.1 Gy) at high dose rate, even with multi-exposure, could not increase tumorigenesis frequency compared to non-irradiated controls. In addition, these results indicate that Fhit could prevent high-dose induced tumorigenesis.

Discussion

Low dose radiation exposure does not increase the tumorigenesis frequency

Our data support that low dose radiation (≤ 0.1 Gy) does not increase tumorigenesis although high dose radiation promotes carcinogenesis, which is believed to be linked to low dose radiation induced adaptive response that

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could protect cells from high dose radiation-induced killing. Such adaptive responses must promote repairing DNA double strand breaks (DSBs) because DSBs are the severest damage for radiation induced mammal killing. Two major kinds of DSB repair exist in mammalian cells, homologous recombination repair (HRR), and non-homologous end-joining (NHEJ). The NHEJ pathway requires Ku80, Ku70, DNA-dependent protein kinase catalytic subunit (DNA-PKcs), ligase IV, XRCC4 and Artemis. The HRR pathway requires Rad51, Rad52, Rad54 as well as the Rad51 paralogs including XRCC2, XRCC3, Rad52B, Rad51C, and Rad51D. The NHEJ pathway is not only involved in DNA repair but is also involved in the immuneresponse through regulating the variable diversity joining V(D)J recombination [17-19]. The V(D)J recombination is important for generating a diverse repertoire of T cell receptor (TCR) and immunoglobulin (Ig) molecules that are necessary for the recognition of diverse antigens including dysfunctional cells such as tumor cells [20]. It is evident that low dose radiation stimulates immunal response, which plays a positive role in preventing carcinogenesis [21]. The detailed mechanism by which low dose radiation stimulates the repair pathways needs to be elucidated in future studies.

Fhit prevents high-dose radiation promoted tumorigenesis

Since Fhit was cloned from the chromosome fragile site 3p14.2 in 1996 [22], the effects of Fhit on tumor development have been widely studied [13, 23-31]. All these data support that Fhit plays a role in preventing tumor development. Our data in this study showed that Fhit could prevent high dose radiation promoted tumorigenesis. Combining these data with our previous published data that Fhit could prevent human cells from high-doses of UV and ionizing radiation-induced *HPRT* mutation [15, 16], we can conclude that Fhit plays an important role in preventing DNA damage promoted tumorigenesis. The mechanism related to Fhit preventing tumorigenesis might involve its role in promoting apoptosis [31], maintaining normal checkpoint response [32, 33], regulating Cyclophilin A [34], and the Akt transduction pathway [35]. However, at this moment, we still do not understand the exact mechanism by which Fhit prevents high-dose radiation induced mutation [16] and tumori-

genesis, which needs more studies in the future.

Taken together, our results indicate that low-dose radiation (0.1 Gy) at high dose rate, even with multi-exposure, would not increase the tumorigenesis, and Fhit could protect high-dose radiation-induced tumorigenesis but has no effect in a low-dose environment.

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References

- [1] Olivieri G, Bodycote J and Wolff S. Adaptive response of human lymphocytes to low concentrations of radioactive thymidine. *Science* 1984; 223: 594-597.
- [2] Sanderson BJ and Morley AA. Exposure of human lymphocytes to ionizing radiation reduces mutagenesis by subsequent ionizing radiation. *Mut. Res.* 1986; 164: 347-351.
- [3] Laval F. Pretreatment with oxygen species increases the resistance of mammalian cells to hydrogen peroxide and g-rays. *Mut. Res.* 1988; 201: 73-79.
- [4] Schäppi-Büchi C. On the genetic background of the adaptive response to X-rays in *Drosophila melanogaster*. *Int. J. Radiat. Biol.* 1994; 65: 427-435.
- [5] Zhou PK, Xiang XQ, Sun WZ, Liu XY, Zhang YP and Wei K. Adaptive response to mutagenesis and its molecular basis in a human T-cell leukemia line primed with a low dose of gamma-rays. *Radiat Environ Biophys.* 1994; 33: 211-217.
- [6] Sasaki MS. On the reaction kinetics of the radioadaptive response in cultured mouse cells. *Int. J. Radiat. Biol.* 1995; 68: 281-291.
- [7] Rigaud O, Laquerbe A and Moustacchi E. DNA sequence analysis of HPRT- mutants induced in human lymphoblastoid cells adapted to ionizing radiation. *Radiat. Res.* 1995; 144: 181-189.
- [8] Ueno AM, Vannais DB, Gustafson DL, Wong JC and Waldren CA. A low, adaptive dose of gamma-rays reduced the number and altered the spectrum of S1- mutants in human-hamster hybrid AL cells. *Mut. Res.* 1996; 358: 161-169.
- [9] Azzam EI, Toledo SMD, Raaphorst GP and Mitchell REJ. Low-dose ionizing radiation decreases the frequency of neoplastic transformation

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- to a level below the spontaneous rate in C3H 10T1/2 Cells *Radiat. Res.* 1996; 146: 369-373.
- [10] Broome EJ, Brown DL and Mitchel REJ. Dose responses for adaptation to low doses of (60)Co gamma rays and (3)H beta particles in normal human fibroblasts *Radiat. Res.* 2002; 158: 181-186.
- [11] Mitchel REJ, Jackson JS, McCann RA and Boreham DR. The adaptive response modifies Latency for radiation-induced myeloid leukemia in CBA/H Mice *Radiat. Res.* 1999; 152: 273-279.
- [12] Sakai K, Iwasaki T, Hoshi Y, Ina Y, Fujita K, Nomura T, Tanooka H, Bing W and Hayata I. Adaptive responses to low dose or low dose-rate irradiation in mice., in, Fifty-third Annual Meeting of the Radiation Research Society, Philadelphia, PA, USA, 2006, pp. 18.
- [13] Huebner K and Croce CM. Cancer and the FRA3B//FHIT fragile locus: it's a HIT. *Br J Cancer* 2003; 88: 1501-1506.
- [14] Fong LYY, Fidanza V, Zanesi N, Lock, LF, Siracusa LD, Mancini R, Siprashvili Z, Ottey M, Martin SE, Druck T, McCue PA, Croce CM and Huebner K. Muir-torre-like syndrome in Fhit-deficient mice. *Proc Nati Acad Sci, USA* 2000; 97: 4742-4747.
- [15] Ottey M, Han S-Y, Druck T, Barnoski B, Croce CM, Fairchild C, Wang Y and Huebner K. Fhit deficient normal and cancer cells are mitomycin C and UVC resistant. *Brit. J. Cancer* 2004; 91: 1669-1677.
- [16] Lu L, Hu B, Yu F and Wang Y. Low dose radiation-induced adaptive response preventing HPRT mutation is Fhit independent *Int. J. Radiat. Biol.* 2009; 85: 532-537.
- [17] Jeggo PA. DNA Breakage and Repair. *Advances Gen.* 1998; 38: 186-218.
- [18] Jackson SP. Sensing and repairing DNA double-strand breaks. *Carcinogenesis.* 2002; 23: 687-696.
- [19] Ma Y, Pannicke U, Schwarz K and Lieber MR. Hairpin Opening and overhang processing by an artemis/DNA-dependent protein kinase complex in nonhomologous end joining and V(D)J recombination. *Cell* 2002; 108: 781-794.
- [20] Abbas A, Lichtman A and Pillai S. *Cellular and Molecular Immunology.* 7th Edition, Publisher: W.B. Saunders Co. 2010.
- [21] Liu S-Z. Cancer control related to stimulation of immunity by low-dose radiation. *Dose Response.* 2007; 5: 39-47.
- [22] Ohta M, Inoue H, Cotticelli MG, Kastury K, Baffa R, Palazzo J, Siprashvili Z, Mori M, McCue P, Druck T, Croce CM and Huebner K. The FHIT gene, spanning the chromosome 3p14.2 fragile site and renal carcinoma-associated t(3;8) breakpoint, is abnormal in digestive tract cancer. *Cell* 1996; 84: 587-597.
- [23] Huebner K and Croce CM. FRA3B and other common fragile sites: the weakest links. *Nature Reviews Cancer* 2001; 1: 214-221.
- [24] Zanesi N, Pekarsky Y and Croce CM. A mouse model of the fragile gene FHIT: From carcinogenesis to gene therapy and cancer prevention. *Mut. Res.* 2005; 591: 103-109.
- [25] Deng Y, Zhou D and Lu Y. Frequent allelic loss at the FRA3B site in endemic nasopharyngeal carcinoma: association with clinical features and Epstein-Barr virus infection. *J Laryngol Otol* 2007; 121: 1073-1078.
- [26] Siraj A, Ibrahim M, Al-Rasheed M, Bu R, Bavi P, Jehan Z, Abubaker J, Murad W, Al-Dayel F, Ezzat A, El-Solh H, Uddin S and Al-Kuraya K. Genetic polymorphisms of methylenetetrahydrofolate reductase and promoter methylation of MGMT and FHIT genes in diffuse large B cell lymphoma risk in Middle East. *Ann Hematol* 2007; 86: 887-895.
- [27] Baker A, Cecener G, Tunca B, Guler G, Egeli U and Tolunay, S. Investigation of mutations and expression of the FHIT gene in Turkish patients with brain metastases derived from non-small cell lung cancer. *Tomori* 2007; 93: 604-607.
- [28] Levin A, Ray A, Zuhlke K, Douglas J and Cooney K. Association between germline variation in the FHIT gene and prostate cancer in Caucasians and African Americans. *Cancer Epidemiol Boimarkers Prev* 2007; 16: 1294-1297.
- [29] Fisher D and McLennan A. Correlation of intracellular diadenosine triphosphate (Ap3A) with apoptosis in Fhit-positive HEK293 cells. *Cancer Lett* 2008; 259: 186-191.
- [30] Yasugi A, Yashima K, Hara A, Koda M, Kawaguchi K, Harada K, Andachi H and Murawaki Y. Fhit, Mlh1, P53 and phenotypic expression in the early stage of colorectal neoplasms. *Oncol Rep* 2008; 19: 41-47.
- [31] Rimessi A, Marchi S, Fotino C, Romagnoli A, Huebner K, Croce CM, Pinton P and Rizzuto R. Intramitochondrial calcium regulation by the FHIT gene product sensitizes to apoptosis. *Proc Nati Acad Sci USA* 2009; 106: 12753-12758.
- [32] Hu B, Han S-Y, Wang X, Ottey M, Potoczek MB, Dicker A, Huebner K and Wang Y. Involvement of the Fhit gene in the ionizing radiation-activated ATR/CHK1 pathway. *J Cell Phys.* 2005; 202: 518-523.
- [33] Ishii H, Wang Y and Huebner K. A Fhit-ing role in the DNA damage checkpoint response. *Cell Cycle* 2007; 6: 1044-1048.
- [34] Semba S and Huebner K. Protein expression profiling identifies cyclophilin A as a molecular target in Fhit-mediated tumor suppression. *Mol Cancer Res* 2006; 4: 529-538.
- [35] Semba S, Trapasso F, Fabbri M, McCorkell KA, Volinia S, Druck T, Iliopoulos D, Pekarsky Y, Ishii H, Garrison PN, Barnes LD, Croce CM and Huebner K. Fhit modulation of the Akt-survivin pathway in lung cancer cells: Fhit-tyrosine 114 (Y114) is essential. *Oncogene* 2006; 25: 2860-2872.

