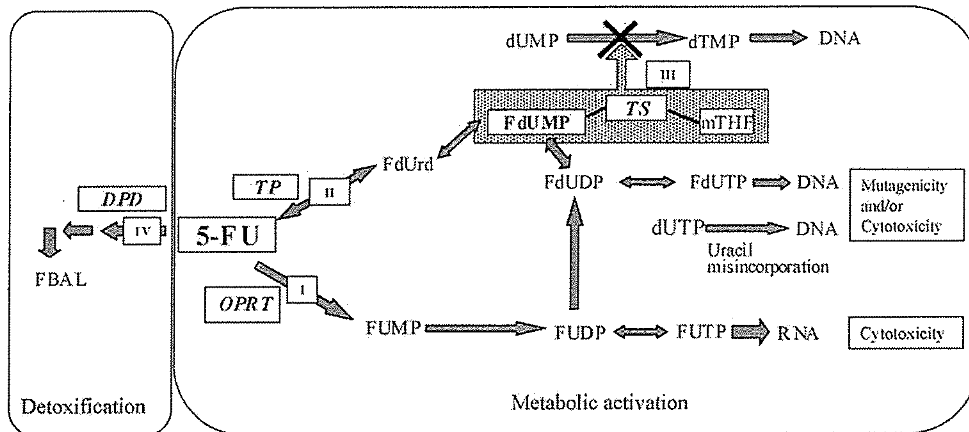

Fig. 5. The intracellular metabolism and catabolism of 5-FU. (I) 5-FU is metabolized by OPRT to FUMP. (II) 5-FU is metabolized to FdUrd by TP, but TP can convert FdUrd to 5-FU. (III) When 5-FU is converted to the active form of 5-FU, FdUMP, it forms complex with TS and 5,10-methylene tetrahydrofolate, and competitively inhibits DNA synthesis. (IV) 5-FU is detoxicated by DPD to FBAL. Thymidine depletion is a trigger for DSBs. DSBs are caused by the misincorporation of dUTP and/or misrepair of the uracil-containing lesion. Furthermore, 5-FU induces DNA strand breaks by the direct incorporation of 5-fluoro-deoxyuridine triphosphate (FdUTP). 5-FU is also misincorporated to RNA, and show the cytotoxicity. Modified from IARC Monographs [26].

genicity in MOLY and WTK-1 cells. Cell cycle analysis showed no clear difference between MOLY cells and the human cell lines, suggesting that p53 gene status did not directly determine the sensitivity of the cells to 5-FU mutagenicity and cytotoxicity.

Fig. 5 shows the intracellular metabolism and detoxification of 5-FU. When 5-FU, a fluorinated pyrimidine base analogue, is converted to 5-fluoro-2-deoxyuridylate monophosphate (FdUMP), it complexes with TS and 5,10-methylene tetrahydrofolate (CH_2FH_4), and competitively inhibits DNA synthesis [23–26]. Thymidine depletion by TS inhibition induces a nucleotide pool imbalance and the misincorporation of dUTP and/or misrepair of the uracil-containing lesion, causing double strand DNA breaks (DSBs) [40–42]. Furthermore, the direct incorporation of 5-fluoro-deoxyuridine triphosphate (FdUTP) inhibits DNA synthesis [24]. Therefore, the intracellular metabolism of 5-FU to FdUMP or FdUTP leads to cytotoxic and mutagenic effects.

5-FU can be metabolized by OPRT to fluoro-uridylate monophosphate and detoxified by DPD to fluoro-β-alanine, or by TP to 5-fluoro-deoxyuridine (FdUrd) and, in turn, back to 5-FU (Fig. 5). In this study, DPD activity was lower and OPRT activity was higher in MOLY cells than in TK6 and WTK-1 cells, suggesting the efficient metabolism of 5-FU in MOLY cells, which could have led to the strong cytotoxic and mutagenic effects that we observed.

Only MOLY cells had no TP activity in this study. TP and OPRT are involved in the intracellular metabolism of 5-FU. Inhibition of OPRT decreases the incorporation of 5-FU into nucleotides and therefore its cytotoxicity, while TP has no effect [43]. Thus, the absence of TP activity in MOLY cells would have little effect on its sensitivity to 5-FU. TS content, on the other hand, was not associated with the cytotoxic and mutagenic effects of 5-FU.

From these findings, we conclude that the difference in DPD and OPRT activity in MOLY, TK6, and WTK-1 cells were responsible for differences in the cells' cytotoxic and mutagenic responses to 5-FU.

The relationship between sensitivity to 5-FU and the intracellular distribution of 5-FU metabolic enzymes indicate that differences in the way that human cells and animal cells metabolize a chemical can cause differences in mutagenicity test results. Thus, potentially mutagenic chemicals should be tested in human cells as well as animal cells.

Compared with WTK-1 cells, TK6 cells had much lower DPD activity, and 5-FU in TK6 cells was efficiently metabolized to FdUMP without being detoxified. But the TS content was higher in TK6 cells than in WTK-1 cells, so 5-FU metabolism would not be very different in TK6 and WTK-1 cells. In this study, however, 5-FU cytotoxicity was higher in TK6 cells than in WTK-1 cells, perhaps because 5-FU is more cytotoxic in p53 normal cells than in p53 mutant or null cells [15–17]. Our results suggest that if 5-FU metabolizing enzyme activity is equivalent, p53 status might influence 5-FU cytotoxicity. Indeed, p53 status might influence responses to cytotoxicity induced by 5-FU as it does to cytotoxicity induced by X-rays [3]. Furthermore 5-FU increased the

MN frequency in MOLY and WTK-1 cells but not TK6 cells, while the ratio of apoptosis cells induced by 5-FU was the highest in TK6 cells. In TK6 cells, the wild type p53 gene might suppress the induction of micronuclei by leading of DNA damaged cells to apoptosis. Thus, 5-FU mutagenicity might be influenced by also the p53 gene status. However, IC_{50} of RS0 was obviously lower and MF was obviously higher in MOLY cells than TK6 and WTK-1 cells. Therefore we suspected that the difference in 5-FU metabolism influenced to 5-FU cytotoxicity and mutagenicity more than the p53 gene status.

In summary, our study showing that MOLY cells were more sensitive than WTK-1 cells to 5-FU cytotoxicity and mutagenicity even though both have a mutated p53 gene suggested that those differences were attributable to differences in 5-FU metabolism rather than the p53 gene status.

Acknowledgments

We thank Drs. Tanaka and Wakuri, Hatano Research Institute, Food and Drug Safety Center, for providing us with MOLY, TK6, and WTK-1 cell lines. We are indebted to Dr. M. Bloom for the precise review of the manuscripts.

References

[1] T.J. Oberly, B.J. Bewsey, G.S. Probst, An evaluation of L5178YTK$^{+/-}$ mouse lymphoma forward mutation assay using 42 chemicals, Mutat. Res. 125 (1984) 291–306.

[2] P. Clay, M.F. Cross, Microwell mutation assays: evaluation of ethyl methanesulfonate, benzo[a]pyrene and benzidine using the tk locus in L5178Y mouse lymphoma cells, Mutagenesis 5 (Suppl.) (1990) 45–54.

[3] M. Honma, M. Hayashi, T. Sofuni, Cytotoxic and mutagenic responses to X-rays and chemical mutagens in normal and p53-mutated human lymphoblastoid cells, Mutat. Res. 374 (1997) 89–98.

[4] M. Honma, L.S. Zhang, M. Hayashi, K. Takeshita, Y. Nakagawa, N. Tanaka, T. Sofuni, Illegitimate recombination leading to allelic loss and unbalanced translocation in p53-mutated human lymphoblastoid cells, Mol. Cell. Biol. 17 (1997) 4774–4781.

[5] M. Honma, M. Hayashi, H. Shimada, N. Tanaka, S. Wakuri, T. Awogi, K.I. Yamamoto, N. Kodani, Y. Nishi, M. Nakadate, T. Sofuni, Evaluation of the mouse lymphoma tk assay (microwell method) as an alternative to the in vitro chromosomal aberration test, Mutagenesis 14 (1999) 5–22.

[6] D. Clive, J.F.S. Spector, Laboratory procedure assessing specific locus mutation at the TK locus in cultured L5178Y mouse lymphoma cells, Mutat. Res. 31 (1975) 17–29.

[7] P.M. Leong-Morgenthaler, C.O.H. Velt, E. Jaccaud, R.J. Turesky, Mutagenicity of 2-amino-3-methylimidazo[4,5-f]quinoline in human lymphoblastoid cells, Carcinogenesis 19 (1998) 1749–1754.

[8] Y.Y. Chuang, Q. Chen, J.P. Brown, J.M. Sedivy, H.L. Liber, Radiation-induced mutations at the autosomal thymidine kinase locus are not elevated in p53-null cells, Cancer Res. 59 (1999) 3073–3076.

[9] R.F. Branda, J.P. O'Neill, E.M. Brooks, L.M. Trombley, J.A. Nicklas, The effect of folate deficiency on the cytotoxic and mutagenic responses to ethyl methanesulfonate in human lymphoblastoid cell lines that differ in p53 status, Mutat. Res. 473 (2001) 51–71.

[10] L. Bassi, M. Carloni, E. Fonti, N.P. de la Pena, R. Meschini, F. Palitti, Pifithrin-alpha, an inhibitor of p53, enhances the genetic instability induced by etoposide (VP16) in human lymphoblastoid cells treated in vitro, Mutat. Res. 499 (2002) 163–176.

[11] S.A. Amundson, F. Xia, K. Wolfson, H.L. Liber, Different cytotoxic and mutagenic responses induced by X-rays in two human lymphoblastoid cell lines derived from a single donor, Mutat. Res. 286 (1993) 233–241.

[12] F. Xia, X. Wang, Y.H. Wang, N.M. Tsang, D.W. Yandell, K.T. Kelsey, H.L. Liber, Altered p53 status correlates with differences in sensitivity to radiation-induced mutation and apoptosis in two closely related human lymphoblast lines, Cancer Res. 55 (1995) 12–15.

[13] S.M. Morris, A role for p53 in the frequency and mechanism of mutation, Mutat. Res. 511 (2002) 45–62.

[14] J.L. Schwartz, R. Jordan, B.A. Sedita, M.J. Swenningson, J.P. Banath, P.L. Olive, Different sensitivity to cell killing and chromosome mutation induction by gamma rays in two human lymphoblastoid cell lines derived from a single donor: possible role of apoptosis, Mutagenesis 10 (1995) 227–233.

[15] D. Arango, G.A. Corner, S. Wadler, P.J. Catalano, L.H. Augenlicht, c-myc/p53 interaction determines sensitivity of human colon carcinoma cells to 5-fluorouracil in vitro and in vivo, Cancer Res. 61 (2001) 4910–4915.

[16] J.L. Grem, K.D. Danenberg, K. Behan, A. Parr, L. Young, P.V. Danenberg, D. Nguyen, J. Drake, A. Monks, C.J. Allegra, Thymidine kinase, thymidylate synthase, and dihydropyrimidine dehydrogenase profiles of cell lines of the National Cancer Institute's Anticancer Drug Screen, Clin. Cancer Res. 7 (2001) 999–1009.

[17] D.B. Longley, J. Boyer, W.L. Allen, T. Latif, P.R. Ferguson, P.J. Maxwell, U. McDermott, M. Lynch, D.P. Harkin, P.G. Johnston, The role of thymidylate synthase induction in modulating p53-regulated gene expression in response to 5-fluorouracil and antifolates, Cancer Res. 62 (2002) 2644–2649.

[18] S.K. Greenwood, M.J. Armstrong, R.B. Hill, C.I. Bradt, T.E. Johnson, C.A. Hilliard, S.M. Galloway, Fewer chromosome aberrations and earlier apoptosis induced by DNA synthesis inhibitors, a topoisomerase II inhibitor or alkylating agents in human cells with normal compared with mutant p53, Mutat. Res. 401 (1998) 39–53.

[19] T. Allio, E.M. Donner, R.J. Preston, A comparison of the roles of p53 mutation and AraC inhibition in the enhancement of bleomycin-induced chromatid aberrations in mouse and human cells, Mutat. Res. 447 (2000) 227–237.

[20] T. Allio, R.J. Preston, Increased sensitivity to chromatid aberration induction by bleomycin and neocarzinostatin results from alterations in a DNA damage response pathway, Mutat. Res. 453 (2000) 5–15.

[21] L.S. Zhang, M. Honma, M. Hayashi, T. Suzuki, A. Matsuoka, T. Sofuni, A comparative study of TK6 human lymphoblastoid and L5178Y mouse lymphoma cell lines in the in vitro micronucleus test, Mutat. Res. 347 (1995) 105–115.

[22] R.D. Storer, A.R. Kraynak, T.W. McKelvey, M.C. Elia, T.L. Goodrow, J.G. DeLuca, The mouse lymphoma L5178Y $Tk^{+/-}$ cell line is heterozygous for a codon 170 mutation in the p53 tumor suppressor gene, Mutat. Res. 373 (1997) 157–165.

[23] A.A. Piper, R.M. Fox, Biochemical basis for the differential sensitivity of human T- and B-lymphocyte lines to 5-fluorouracil, Cancer Res. 42 (1982) 3753–3760.

[24] R.B. Diasio, B.E. Harris, Clinical pharmacology of 5-fluorouracil, Clin. Pharmacokinet. 16 (1989) 215–237.

[25] D.M. Thomas, J.R. Zalcberg, 5-fluorouracil: a pharmacological paradigm in the use of cytotoxics, Clin. Exp. Pharmacol. Physiol. 25 (1998) 887–895.

[26] 5-fluorouracil, IARC Monogr. Eval. Carcinogen. Risk Chem. Hum. 26 (1981) 217–235.

[27] L. Griffiths, I.J. Stratford, Platelet-derived endothelial cell growth factor thymidine phosphorylase in tumor growth and response to therapy, Br. J. Cancer 76 (1997) 689–693.

[28] N. Yajima, K. Kondo, K. Morita, Reverse mutation tests in *Salmonella typhimurium* and chromosomal aberration tests in mammalian cells in culture on fluorinated pyrimidine derivatives, Mutat. Res. 88 (1981) 241–254.

[29] A. Ohuchida, A. Furukawa, J. Yoshida, M. Watanabe, F. Aruga, Y. Miwa, K. Shinkawa, N. Kinae, Micronucleus assays on 5-fluorouracil and 6-mercaptopurine with mouse peripheral blood reticulocytes, Mutat. Res. 278 (1992) 139–143.

[30] T. Sofuni, M. Honma, M. Hayashi, H. Shimada, N. Tanaka, S. Wakuri, T. Awogi, K.I. Yamamoto, Y. Nishi, M. Nakadate, Detection of in vitro clastogens and spindle poisons by the mouse lymphoma assay using the microwell method: interim report of an international collaborative study, Mutagenesis 11 (1996) 349–355.

[31] 5-fluorouracil, IARC Monogr. Eval. Carcinogen. Risk Chem. Hum. (Suppl. 6) (1987) 316–318.

[32] T. Matsushima, M. Hayashi, A. Matsuoka, M. Ishidate, K.F. Miura, H. Shimizu, Y. Suzuki, K. Morimoto, H. Ogura, K. Mure, K. Koshi, T. Sofuni, Validation study of the in vitro micronucleus test in a Chinese hamster lung cell line (CHL/IU), Mutagenesis 14 (1999) 569–580.

[33] Y. Gavrieli, Y. Sherman, S.A. Ben-Sasson, Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation, J. Cell Biol. 119 (1992) 493–501.

[34] M. Carbonari, M. Cibati, M. Fiorilli, Measurement of apoptotic cells in peripheral blood, Cytometry 22 (1995) 161–167.

[35] C.P. Spears, A.H. Shahinian, R.G. Moran, C. Heidelberger, T.H. Corbett, In vivo kinetics of thymidylate synthetase inhibition of 5-fluorouracil-sensitive and -resistant murine colon adenocarcinomas, Cancer Res. 42 (1982) 450–456.

[36] T. Takechi, J. Uchida, F.A.M. Fukushima, Enhancing 5-fluorouracil cytotoxicity by inhibiting dihydropyrimidine dehydrogenase activity with uracil in human tumor cells, Int. J. Oncol. 11 (1997) 1041–1044.

[37] F.N. Naguib, M.H. el Kouni, S. Cha, Enzymes of uracil catabolism in normal and neoplastic human tissues, Cancer Res. 45 (1985) 5405–5412.

[38] G.J. Peters, E. Laurensse, A. Leyva, J. Lankelma, H.M. Pinedo, Sensitivity of human, murine, and rat cells to 5-fluorouracil and 5′-deoxy-5-fluorouridine in relation to drug-metabolizing enzymes, Cancer Res. 46 (1986) 20–28.

[39] Y. Maehara, H. Nakamura, Y. Nakane, K. Kawai, M. Okamoto, S. Nagayama, T. Shirasaka, S. Fujii, Activities of various enzymes of pyrimidine nucleotide and DNA syntheses in normal and neoplastic human tissues, Gann 73 (1982) 289–298.

[40] H.A. Ingraham, B.Y. Tseng, M. Goulian, Nucleotide levels and incorporation of 5-fluorouracil and uracil into DNA of cells treated with 5-fluorodeoxyuridine, Mol. Pharmacol. 21 (1982) 211–216.

[41] N.J. Curtin, A.L. Harris, G.W. Aherne, Mechanism of cell death following thymidylate synthase inhibition: 2′-deoxyuridine-5′-triphosphate accumulation, DNA damage, and growth inhibition following exposure to CB3717 and dipyridamole, Cancer Res. 51 (1991) 2346–2352.

[42] R.D. Ladner, The role of dUTPase and uracil-DNA repair in cancer chemotherapy, Curr. Protein Pept. Sci. 2 (2001) 361–370.

[43] M. Fukushima, Y. Murakami, N. Suzuki, K. Aiba, The analysis of the innate pathways of 5-fluorouracil phosphorylation in human gastrointestinal cancer cell lines in vitro and in vivo, Oncol. Rep. 4 (1997) 1189–1194.