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Gene Expression Profiling of Doxifluridine Treated Liver, Small and Large Intestine in Cynomolgus (Macaca fascicularis) Monkeys

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Abstract

The mechanism of cytotoxicity of doxifluridine, a prodrug fluorouracil (5-FU), has been ascribed to the misincorporation of fluoropyrimidine into RNA and DNA and to the inhibition of the nucleotide synthetic enzyme thymidylate synthase. Increased understanding of the mechanism of 5-FU has led to the development of strategies that increases its anticancer activity or predicts its sensitivity to patients. Using GeneChip® Rhesus Macaque Genome arrays, we analyzed gene expression profiles of doxifluridine after two weeks repeated administration in cynomolgus monkey. Kegg pathway analysis suggested that cytoskeletal rearrangement and cell adhesion remodeling were commonly occurred in colon, jejunum, and liver. However, expression of genes encoding extracellular matrix was distinguished colon from others. In colon, COL6A2, COL18A1, ELN, and LAMA5 were over-expressed. In contrast, genes included in same category were down-regulated in jejunum and liver. Interestingly, MMP7 and TIMP1, the key enzymes responsible for ECM regulation, were over-expressed in colon. Several studies were reported that both gene reduced cell sensitivity to chemotherapy-induced apoptosis. Therefore, we suggest they have potential as target for modulation of 5-FU action. In addition, the expression of genes which have been previously known to involve in 5-FU pathway, were examined in three organs. Particularly, there were more remarkable changes in colon than in others. In colon, ECGF1, DYPD, TYMS, DHFR, FPGS, DUT, BCL2, BAX, and BAK1 except CAD were expressed in the direction that was good response to doxifluridine. These results may provide that colon is a prominent target of doxifluridine and transcriptional profiling is useful to find new targets affecting the response to the drug.

Keywords: Doxifluridine, Gene expression, Liver, Intestine, 5-FU

The fluoropyrimidine, 5-FU is widely used in the treatment of a range of cancers, including breast and gastric cancers, and particularly colorectal cancer. As 5-FU mimics uracil, it interferes with nucleic acid synthesis as incorporating into DNA or RNA instead of uracil, and thus slow tumor growth. To achieve its pharmacologic effects, 5-FU must be activated by intermediate metabolism. Therefore, the expression of 5-FU converting enzymes had important implications for drug efficacy. So far, initial key enzymes in the 5-FU metabolic pathway such as thymidylate synthase, thymidine phosphorylase and dihydropyrimidine dehydrogenase have been reported to be associated with drug sensitivity[1-3].

Although 5-FU based on chemotherapy improved its anticancer activity in combination with other chemotherapies such as irinotecan and oxaliplatin, drug resistance remains an important obstacles to the clinical use of 5-FU[4,5]. Drug resistance to fluoropyrimidines is a multifactorial event that includes transport mechanism, metabolism, molecular mechanism, protection from apoptosis and resistance via cell cycle kinetics[6]. To comprehend drug efficacy or resistance, it is an essential step to understanding the mechanisms by which the drug caused toxicity. Furthermore, to gain a deeper insight into the events responsible for drug action, more comprehensive investigations are still needed.

In order to identify and measure changes in gene expression, we employed the GeneChip® enabling a gene expression study on a genome wide scale. Using this technology, we investigated numerous molecular event occurred after administration of doxifluridine, a prodrug of 5-FU as well as the expression of enzymes involved in the 5-FU metabolic pathway. We compared the patterns of gene expression after administration of 5-FU in liver, small (jejunum) and large intestine (colon) of cynomolgus monkeys and found

Table 1. Highest-ranking pathways of the doxifluridine response based on the KEGG library.

Rank	Large Intestine		Small Intestine		Liver	
	KEGG Pathway	Gene	KEGG Pathway	Gene	KEGG Pathway	Gene
1	MAPK signaling pathway	104	MAPK signaling pathway	7	MAPK signaling pathway	8
2	Focal adhesion	94	Focal adhesion	7	Regulation of actin cytoskeleton	7
3	Regulation of actin cytoskeleton	86	Cytokine-cytokine receptor interaction	7	Adherens junction	7
4	Insulin signaling pathway	64	ECM-receptor interaction	6	Linoleic acid metabolism	6
5	Wnt signaling pathway	56	Cell Communication	6	Jak-STAT signaling pathway	6
6	Gap junction	54	Regulation of actin cytoskeleton	5	Focal adhesion	6
7	Cytokine-cytokine receptor interaction	54	Neuroactive ligand-receptor interaction	5	Pathogenic Escherichia coli infection-EPEC	5
8	Natural killer cell mediated cytotoxicity	51	TGF-beta signaling pathway	4	Pathogenic Escherichia coli infection-EHEC	5
9	Axon guidance	51	Metabolism of xenobiotics by cytochrome P450	4	Natural killer cell mediated cytotoxicity	5
10	Leukocyte transendothelial migration	50	Hematopoietic cell lineage	4	Leukocyte transendothelial migration	
11	Cell cycle	50	Bile acid biosynthesis	4	Cytokine-cytokine receptor interaction	5
12	Tight junction	48	Tyrosine metabolism	3	Cell adhesion molecules (CAMs)	5
13	Calcium signaling pathway	46	Type I diabetes mellitus	3	Wnt signaling pathway	4
14	Purine metabolism	45	Purine metabolism	3	Neuroactive ligand-receptor interaction	4
15	GnRH signaling pathway	43	Jak-STAT signaling pathway	3	Antigen processing and presentation	4
16	Cell adhesion molecules (CAMs)	43	Hedgehog signaling pathway	3	gamma-Hexachlorocyclohexane degradation	3
17	Glycan structures-biosynthesis 1	40	Glycerophospholipid metabolism	3	Tight junction	3
18	Apoptosis	40	Complement and coagulation cascades	3	Terpenoid biosynthesis	3
19	Jak-STAT signaling pathway	39	Cell adhesion molecules (CAMs)	3	Metabolism of xenobiotics by cytochrome P450	3
20	Adherens junction	39	Antigen processing and presentation	3	Maturity onset diabetes of the young	3

This table include genes that were changed more than a 2 fold at low or high dose (Welch's *t* test, $P < 0.05$).

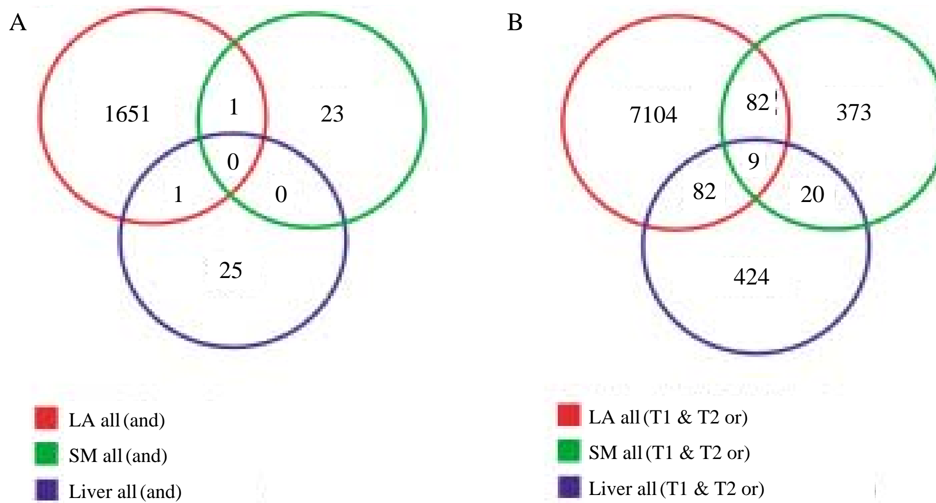

Figure 1. Venn Diagrams of the significantly changed genes in three organs. A. Genes that were selected based on minimum 2 fold change at both dose, B. Genes that were selected based on minimum 2 fold change at low or high dose (Welch's t test, $P < 0.05$). LA: large intestine, Sm: small intestine.

that colon is a main susceptible organ to 5-FU cytotoxicity. This study might provide potential opportunities to identify novel genes for response to 5-FU based chemotherapy.

The differentially expressed genes (DEGs) were selected based on at a minimum 2 fold change at low or high dose and Welch's t test $P < 0.05$ were considered as statistical significance. In this analysis, we identified 7277, 484, and 535 genes at large intestine, small intestine, and liver, respectively. As compare with other organs, the number of the changed genes in large intestine was more than 10 times, which might reflect that large intestine is a prominent target of doxifluridine. For compare gene expression pattern in different organs, Venn diagram analysis was performed. As Figure 1B, commonly changed genes at both dose levels in three organs were not found. Moreover, only 9 genes were commonly regulated at low or high dose (Figure 1A). This analysis would seem to represent that the regulation of genes by doxifluridine is not correlated between three organs.

In the next step of the analysis, we used 'a non a priori' approach to prioritize candidate pathways that were affected by doxifluridine. We matched the DEGs to the pathway database of the KEGG (Kyoto Encyclopedia of Genes and Genomes). Highest 20 ranking of total gene counts were showed in Table 1. Even if same genes were not existed in the same pathways, genes involved in MAPK signaling and Jak-stat signaling pathways were consistently regulated by doxifluridine in all organs. In addition, many genes involved in focal adhesion, cell adhesion molecules, regulation of actin cytoskeleton, and cytokine-cytokine receptor interaction pathway were significantly co-expressed in all organs.

Cell adhesion proteins typically have domains that extend into both the extracellular space and the intracellular space. The intracellular domain binds to protein components of the cell's cytoskeleton. Also, the extracellular domain can bind to other molecules that might be either on the surface of an adjacent cell (cell-to-cell adhesion) or part of the extracellular matrix (cell-to-ECM adhesion). Accordingly, the genes related to extracellular matrix organization were further analyzed. To perform strict analysis, DEGs were selected in large intestine based on statistical significance ($P < 0.01$). In large intestine, collagen, type VI, alpha 2 (*COL6A2*) and collagen, type XVIII, alpha 1 (*COL18A1*) genes encoding collagens that are the most abundant glycoproteins in the ECM were overexpressed. Non-glycoprotein encoding genes, elastin (*ELN*) and laminin, alpha 5 (*LAMA5*) were also up-regulated. Moreover, matrix metallopeptidase 7 and 19 (*MMP17*, *MMP19*) that function in collagen catabolism, and its inhibitor, TIMP metallopeptidase inhibitor 1 (*TIMP1*) were up-regulated. In contrast, the genes included in this category in small intestine and liver were down-regulated (Table 2).

To understanding the mechanisms involved in metabolism of 5-FU, we focused on pyrimidine converting enzymes and other genes coding for proteins with direct relevance to the mechanisms 5-FU (7-9). Among known genes involved in the 5-FU pathway, Figure 2 presented significantly changed genes more than 2 fold in large intestine. *ECGF1, TYMS, FPGS*, and *CAD* were up-regulated but *DYPD, DHFR, DUT*, and *BCL2* were down-regulated. On the other hand, lower expression changes were shown in small intestine and liver except *TYMS* and *DHFR*. This analysis demonstrated that the large intestine is more sensitive than other organ to 5-FU response.

Table 2. Comparison of genes related to extracellular matrix organization.

Probe Set ID	Symbol	Gene title	Fold Change	
			Low	High
Large Intestine				
38899.1.S1_at	*COL6A2*	Collagen, type VI, alpha 2	1.1	1.8
23773.1.S1_at	*COL18A1*	Collagen, type XVIII, alpha 1	0.4	2.6
11341.1.S1_s_at	*ELN*	Elastin	1.0	1.2
6074.1.S1_at	*LAMA5*	Laminin, alpha 5	0.3	1.1
39260.1.S1_at	*FBLN1*	Fibulin 1	1.6	3.6
42196.1.S1_at	*ECM1*	Extracellular matrix protein 1	1.2	2.4
27675.1.S1_at	*MMP7*	Matrix metallopeptidase 7 (matrilysin)	5.5	10.3
5715.1.S1_at	*TIMP1*	TIMP metallopeptidase inhibitor 1	1.5	3.2
17553.1.S1_at	*MMP19*	Matrix metallopeptidase 19	1.0	1.8
7884.1.S1_at	*CRTAP*	Cartilage associated protein	1.2	1.8
7832.1.S1_at	*SPOCK2*	Sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testican) 2	1.2	1.6
11991.1.S1_at	*WNT9A*	Wingless-type MMTV integration site family, member 9A	1.0	1.2
Small Intestine				
26445.1.S1_at	*COL1A1*	Collagen, type I, alpha 1	−0.8	−1.7
23996.1.S1_at	*COL1A2*	Collagen, type I, alpha 2	−0.6	−1.1
10377.1.S1_at	*LAMA4*	Laminin, alpha 4	−0.4	−1.0
31007.1.S1_s_at	*FN1*	Fibronectin 1	−0.9	−1.1
26463.1.S1_at	*MMP28*	Matrix metallopeptidase 28	0.8	1.4
Liver				
23996.1.S1_at	*COL1A2*	Collagen, type I, alpha 2	0.3	−1.6
23114.1.S1_at	*COL3A1*	Collagen, type III, alpha 1	0.4	−1.3
38127.1.S1_at	*FBN2*	Fibrillin 2	1.1	3.0
27675.1.S1_at	*MMP7*	Matrix metallopeptidase 7 (matrilysin)	−1.8	−0.8

This table include genes that were changed more than a 2 fold at low or high dose (Welch's t test, $P<0.01$ for large intestine, $P<0.05$ for small intestine and liver). Fold changes were presented as log base 2.

Discussion

5-FU was catabolized and anabolized by the same biological pathway as naturally occurring pyrimidines and many of the 5-FU metabolic pathway genes was already known[7-9]. Investigation of expression of genes related to metabolic pathway is fundamental to understand the 5-FU toxicity. In brief, doxifluridine is converted to by platelet derived endothelial cell growth factor 1/thymidine phosphorylase (*ECGE1*/*TP*) in tumor and normal tissue[10]. 5-FU is further metabolized to two active metabolites, 5-fluoro-2-deoxyuridine monophosphate (FdUMP) and 5-fluorouridine triphosphate (FUTP). FUTP can be incorporated into RNA in place of uridine triphosphate (UTP), which interferes with RNA processing and protein synthesis. FdUMP and the folate cofactor, 5, 10-methylenetetrahydrofolate (CH_2THF), bind to thymidylate synthase (*TYMS*) to form a covalently bound ternary complex, which subsequently inhibit DNA synthesis. Dihydropyrimidine dehydrogenase (*DYPD*) catalyze the initial, rate limiting step of the catabolism of 5-FU. The present study revealed that large intestine is more sensitive to doxifluridine than others under our experiment condition. Although it is still controversial whether high expressions of *TP*, *TYMS* and low level of *DYPD* are correlated with a good response in colon cancer patients, these are representative enzymes which were mainly discussed as prognostic factors in response to 5-FU[11,12]. Our data were also shown a consistent result as past studies. Methotrexate (MTX) is an antifolate inhibitor of dihydrofolate reductase (*DHFR*), which catalyze the conversion dihydrofolate to tetrahydrofolate (the precursor of CH_2THF). MTX also inhibits *TYMS* indirectly by diminishing levels of the *TYMS* cosubstrate CH_2THF which lead to synergize with 5-FU[7]. Folylpolyglutamate synthetase (FPGS) catalyzes the addition of glutamate to reduced folate (tetrahydrofolate, CH_2THF) to form folate polyglutamates[13]. Both down-regulations of *DHFR* and over-expression *FPGS* may contribute to reduce CH_2THF and then make *TYMS* inactively. dUTP pypohosphatase (*DUT*) mediates conversion of dUTP to dUMP, which can be used by *TYMS* for synthesis of dTMP. Thus, down *DUT* expression might be also against to *TYMS* Pre-apoptotic genes, such as BCL2-antagonist/killer 1 (*BAK1*) and BCL2-associated X

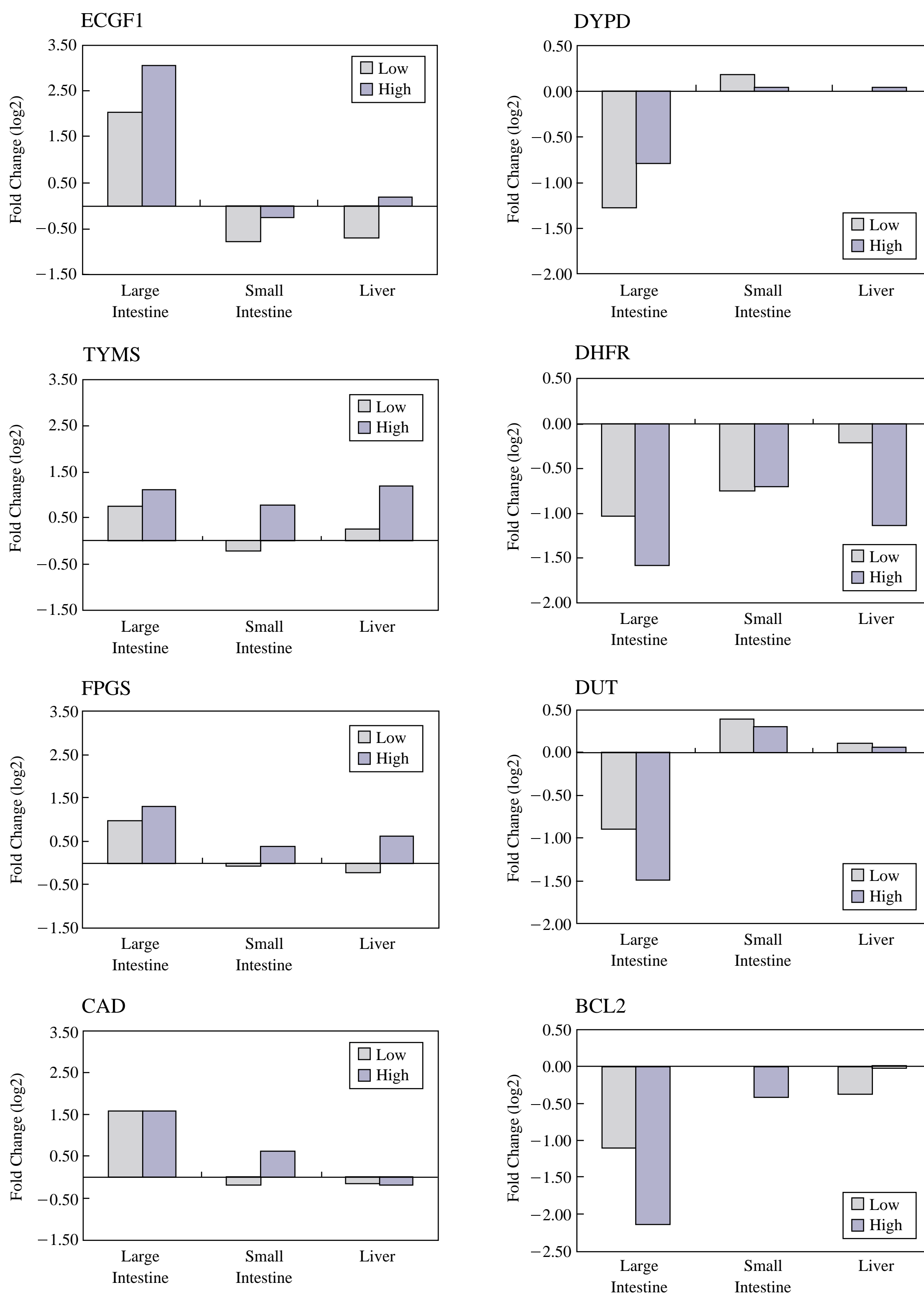

Figure 2. Genes were expressed with relevance to 5-FU pathways. *ECGF1*: platelet derived endothelial cell growth factor 1, *DYPD*: dihydropyrimidine dehydrogenase, *TYMS*: thymidylate synthase, *DHFR*: dihydrofolate reductase, *FPGS*: folylpolyglutamate synthetase, *DUT*: dUTP pyrophosphatase, *CAD*: carbamoylphosphate synthetase 2/aspartate transcarbamoylase/ dihydroorotase, *BCL2*: B-cell CLL/lymphoma 2, *BAX*: BCL2-associated X protein, *BAK1*: BCL2-antagonist/killer.

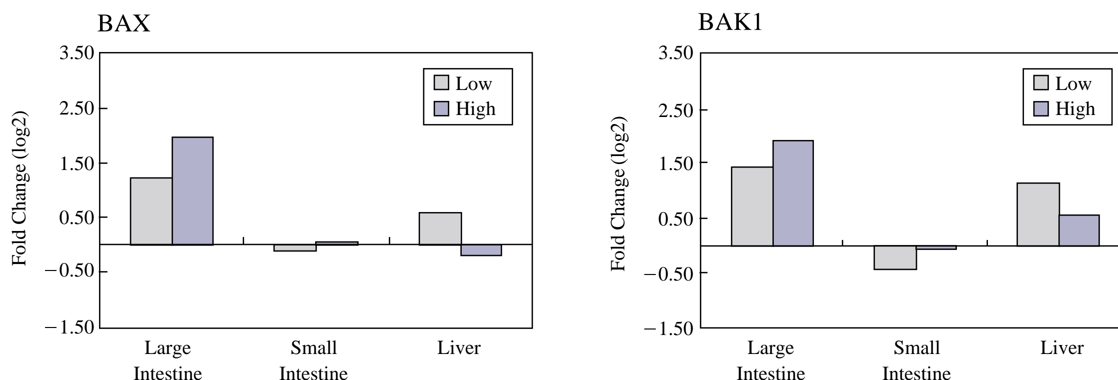

Figure 2. Continued.

protein (*BAX*) were induced and anti-apoptotic B-cell CLL/lymphoma 2 (*BCL2*) was repressed. Above mentioned genes were expressed in the direction that was good response to doxifluridine except *CAD*. *CAD* was expressed in the opposite direction. This gene encodes a trifunctional protein which is associated with the enzymatic activities of the first 3 enzymes in the 6-step pathway of pyrimidine biosynthesis: carbamoylphosphate synthetase (CPS II), aspartate transcarbamoylase, and dihydroorotase. Therefore, overexpression of *CAD* can stimulate *de novo* pyrimidine synthesis and compete with drugs that interact with enzymes of the *de novo* pathway, thereby selectively protecting tissue. Phosphonacetyl-L-aspartate (PALA), which inhibit *CAD* and depleted uridine nucleotide pools *in vitro* and *in vivo*, selectively potentiated the antitumor activity of 5-FU[14].

Pathway analysis revealed that administration of doxifluridine caused the extracellular alterations concerning cell adhesion and cell-matrix interaction as well as corresponding rearrangement of the intracellular cytoskeleton in all organs. In particular, our study led to a noteworthy result in differential expression pattern associated with ECM organization. The extracellular matrix (ECM) holds cells together and maintains the three-dimensional structure of organs. Cell interactions with extracellular matrix (ECM) greatly influence cell survival, and removal of anchorage-dependent cells from their association with the ECM results in apoptotic cell death, known as “anoikis”[15]. Extracellular matrix components were also important in allowing tumor cells to express a growth advantage. As a tumor grows, ECM components either produced by the tumor cells or its stroma.

Over-expression of the genes encoding ECM components, such as *COL6A2, COL18A1, ELN*, and *LAMA5* in large intestine suggested that ECM increase was occurred in response to doxifluridine. The key enzymes responsible for degradation and deposition of all the protein components of extracellular matrix are matrix metalloproteinases (MMPs) and their endogenous inhibitors. Up-regulation of *TIMP1* may contribute ECM increase in large intestine. Although, MMPs activity was known to counteract TIMPs’ action, MMP7 was also up-regulated. In the literature, high expression of MMP7 and TIMP1 has been observed in gastric cancer[16].

MMP7 (matrilysin) and TIMP1 are frequently expressed in various types of cancer including colon, prostate, breast and brain cancers. Several studies demonstrated that the suppression of matrilysin or TIMP1 were associated with low invasiveness and slow tumor growth in colon[17-19]. In addition, both gene expressions reduced tumor cell sensitivity to chemotherapy-induced apoptosis[20,21]. Therefore, present study suggests that MMP7 and TIMP1 have potential as target for modulation of 5-FU action.

Several studies have shown that extracellular matrix regulation reduces chemotherapy-induced apoptosis in cancer cell. In the past study, ECM protects malignant cells from cytotoxic stress via adhesion to integrin receptors[20]. Studies with lymphoma cells showed that these cells expressed increased resistance to etoposide treatment when plated on laminin and in the presence of IL-4[21]. The regulation of ECM on 5-FU action in colon was little known. The down-regulation of heparin/heparan sulfate interacting protein (HIP) was significantly increased in parallel with apoptosis after treatment with 5-FU[22]. HIP, involved in cell-extracelluar matrix interactions, acts through interaction with heparin/heparan sulfate proteoglycans (HSPGs). In our data, over-expression of HSPG2 ($P < 0.05$) suggest that it may disturb cytotoxicity of 5-FU. However, it was reported that stromal ECM components such as collagen I and fibronectin, increased the toxicity of 5-FU for colon cancer cell lines. The

relation between ECM and 5-FU is needed to further study.

In the past, attempts to modulate 5-FU cytotoxicity have focused primarily on increasing activation, decreasing degradation and enhancing *TYMS* inhibition. Using oligonucleotide microarray suggested that ECM alteration was an event in response to doxifluridine. Particularly, MMP7 and TIMP1 expressions which regulated ECM organization are expected to modulate the cytotoxic activity of 5-FU.

Methods

Animal Treatment

Male cynomolgus monkey (*Macaca fascicularis*), approximately 4-6 years old, were kept in a controlled temperature and humidity with a 12-hour light/dark cycle. Animals were randomly assigned to three per group and were administered via oral (p.o) using catheter at 5 mL/kg body weight. Doxifluridine was dissolved in sterilized distilled water and administered once daily up to day 14 at dose of 50 (low) and 100 mg/kg (high). Control animals were received corresponding quantities (5 mL/kg). Monkeys were sacrificed at 24 hr after the last administration. Liver, jejunum (small intestine), and colon (large intestine) were dissected and submerged in RNAlater (Ambion, USA). Samples overnight in the reagent at 4°C and then remove the reagent, and store at −80°C until next processing.

RNA Extraction

Total RNA was isolated using Trizol reagent (Molecular Research Center, Inc., USA) and purified using RNeasy mini kit (Qiagen, Germany) according to manufacturer instructions. Isolated total RNA quantified using NanoDrop® ND-1000 (NanoDrop, USA) and RNA integrity were determined by Agilent Bioanalyzer 2100 (Agilent Technologies, USA).

Microarray Experiments

Affymetrix GeneChip® Rhesus Macaque Genome array containing over 52,024 rhesus probe sets (representing over 47,000 well-substantiated rhesus transcripts) was used for microarray experiment. Target preparation and microarray processing procedures were performed as described in the Affymetrix GeneChip® Expression Analysis Manual (Affymetrix, Inc., USA). Briefly, 10 μg of purified total RNA was used to synthesize double-strand cDNA with SuperScript II and T7-$(dT)_{24}$ primer. Then, biotinylated cRNA was synthesized from the purified double-strand cDNA using the IVT (in vitro transcription) Labeling kit and was purified and fragmented. The fragmented cRNA next hybridized to the GeneChip®, which was washed and stained with streptavidin-phycoerythrin (SAPE). Scanning was performed with a GeneChip® scanner 3000.

Data Analysis

Scanned data (.cel files) were analyzed using GenPlex software version 2.0 (Istech Inc., Korea). Data normalization was performed using global scale normalization. The differentially expressed genes (DEGs) were selected based on statistical significance assigned at a minimum 2-fold change at low or high dose and Welch's t test ($P < 0.05$). In this analysis, we identified 7277, 484, and 535 genes at large intestine, small intestine, and liver, respectively. In addition, the other DEGs that commonly regulated at both doses, which were 1653, 24, and 26 genes respectively, were also selected. To compare gene expression pattern of three organs, Venn diagram analysis was performed using above two DEGs. To prioritize candidate pathways that were affected by doxifluridine, the former DEGs were matched to KEGG pathway database. In the next step, we analyzed the expression of genes which have been previously known to be involved in 5-FU action mechanism[7-9].

Acknowledgements

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References

1. Salonga, D. *et al*. Colorectal tumors responding to 5-fluorouracil have low gene expression levels of dihydropyrimidine dehydrogenase, thymidylate synthase, and thymidine phosphorylase. *Clin. Cancer Res*. **6**: 1322-1327 (2000).
2. Terashima, M. *et al*. Role of thymidine phosphorylase and dihydropyrimidine dehydrogenase in tumor progression and sensitivity to doxifluridine in gastric cancer patients. *Eur. J. Cancer* **38**:2375-2381 (2002).
3. Giacchetti, S. *et al*. Phase III multicenter randomized trial of oxaliplatin added to chronomodulated fluorouracilleucovorin as first-line treatment of metastatic colorectal cancer. *J. Clin. Oncol.* **18**:136-147 (2000).
4. Douillard, J. Y. *et al*. Irinotecan combined with fluorouracil compared with fluorouracil alone as first-line treatment for metastatic colorectal cancer: a multicentre randomised trial. *Lancet* **355**:1041-1047 (2000).

5. Allegra, C. J. *et al*. Investigation of the prognostic and predictive value of thymidylate synthase, p53, and Ki-67 in patients with locally advanced colon cancer. *J. Clin. Oncol.* **20**:1735-1743 (2002).
6. Mader, R. M., Muller, M. & Steger, G. G. Resistance to 5-fluorouracil. *Gen. Pharmacol.* **31**(5):661-666 (1998).
7. Longley, D. B., Harkin, D. P. & Johnston, P. G. 5-fluorouracil: mechanisms of action and clinical strategies. *Nat. Rev. Cancer* **3**:330-338 (2003).
8. Schmidt, W. M. *et al*. Dissecting progressive stages of 5-fluorouracil resistance in vitro using RNA expression profiling. *Int. J. Cancer* **112**(2):200-212 (2004).
9. Kidd, E. A. *et al*. Variance in the expression of 5-Fluorouracil pathway genes in colorectal cancer. *Clin. Cancer Res.* **11**(7):2612-2619 (2005).
10. Kono, A. *et al*. Activation of 5′-deoxy-5-fluorouridine by thymidine phosphorylase in human tumors. *Chemo. Pharm. Bull.* **31**:175-178 (1983).
11. Hasegawa, S. *et al*. Thymidine phosphorylase expression and efficacy of adjuvant doxifluridine in advanced colorectal cancer patients. *Oncol. Rep.* **13**(4):621-626 (2005).
12. Kornmann, M. *et al*. Thymidylate synthase and dihydropyrimidine dehydrogenase mRNA expression levels: predictors for survival in colorectal cancer patients receiving adjuvant 5-fluorouracil. *Clin. Cancer Res.* **9**(11):4116-4124 (2003).
13. Sohn, K. J. *et al*. Effects of folylpolyglutamate synthetase modulation on chemosensitivity of colon cancer cells to 5-fluorouracil and methotrexate. *Gut.* **53**(12):1825-1831 (2004).
14. O'Dwyer, P. J. *et al*. Phase II study of biochemical modulation of fluorouracil by low-dose PALA in patients with colorectal cancer. *Clin. Oncol.* **8**(9):1497-1503 (1990).
15. Boudreau, N., Werb, Z. & Bissell, M. J. Suppression of apoptosis by basement membrane requires three-dimensional tissue organization and withdrawal from the cell cycle. *Proc. Natl. Acad. Sci. USA* **93**(8):3509-3513 (1996).
16. Jinawath, N. *et al*. Comparison of gene-expression profiles between diffuse-and intestinal-type gastric cancers using a genome-wide cDNA microarray. *Oncogene* **23**(40):6830-6844 (2004).
17. Yamamoto, H., Itoh, F., Hinoda, Y. & Imai, K. Suppression of matrilysin inhibits colon cancer cell invasion *in vitro*. *Int. J. Cancer* **61**:218-222 (1995).
18. Miyazaki, K. *et al*. Matrilysin as a target for chemotherapy for colon cancer: use of antisense oligonucleotides as antimetastatic agents. *Cancer Chemother. Pharmacol.* **43**:S52-55 (1999).
19. Ohtani, Y. *et al*. Suppression effects of human recombinant tissue inhibitor of metalloproteinases-1(TIMP-1) on tumor proliferation using *in vivo* treatment model of well-differentiated colon cancer cell line, HT29. *Acta Med. Okayama.* **60**(5):257-266 (2006).
20. Davidsen, M. L. *et al*. TIMP-1 gene deficiency increases tumour cell sensitivity to chemotherapy-induced apoptosis. *Br. J. Cancer* **95**(8):1114-1120 (2006).
21. Fingleton, B., Vargo-Gogola, T., Crawford, H. C. & Matrisian. L. M. Matrilysin [MMP-7] expression selects for cells with reduced sensitivity to apoptosis. *Neoplasia* **3**(6):459-468 (2001).
22. Sethi, T. *et al*. Extracellular matrix proteins protect small cell lung cancer against apoptosis: A mechanism for small cell lung cancer growth and drug resistance *in vivo. Nat. Med*. **5**:662-668 (1999).
23. Taylor, S. T., Hickman, J. A. & Dive, C. Epigenetic determinants of resistance to etoposide regulation of Bcl-X(L) and Bax by tumor microenvironmental factors. *J. Natl. Cancer Inst*. **92**:18-23 (2000).
24. Liu, J. J. *et al*. Heparin/heparan sulfate interacting protein plays a role in apoptosis induced by anticancer drugs. *Carcinogenesis* **25**(6):873-879 (2004).