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Changes to the dihydropyrimidine dehydrogenase gene copy number influence the susceptibility of cancers to 5-FU-based drugs: Data mining of the NCI-DTP data sets and validation with human tumour xenografts

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ABSTRACT

Patient response to the anti-tumour drug 5-fluorouracil (5-FU) is variable, but predicting the response rate is important for the selection of effective chemotherapy. Our aim was to identify alterations in DNA copy number that influence susceptibility of cancer cells to 5-FU-based drugs. The NCI public database was used to identify chromosome loci associated with drug sensitivity and DNA copy number. One of the 11 candidates, the cytogenetic band 1p21.3, harbours the dihydropyrimidine dehydrogenase (*DPD*) gene. To validate this finding, the *DPD* copy number and *in vivo* sensitivity to 5-FU-based drugs were determined in 31 human tumour xenografts. Those xenografts demonstrating low sensitivity had significantly higher *DPD* copy numbers than highly sensitive tumours ($P < 0.002$). Moreover, DPD mRNA expression levels were significantly correlated with *DPD* copy numbers ($P < 0.046$). An assessment of copy number may be a more precise method of predicting the sensitivity of cancer patients to 5-FU related drugs.

1. Introduction

5-Fluorouracil (5-FU) has been the most widely used agent in the treatment of cancers for over 40 years. Although, when used as a single agent, its response rate is less than 20%,[1] chemotherapeutic regimens containing 5-FU play a central role in cancer therapy. Alternative 5-FU-based medication, such as tegafur-oxonate (TS-1) or capecitabine, offers improved response rates, but the response to these drugs is variable among treated patients. It is therefore of great importance to predict the response rate of each individual case in order to reduce inappropriate chemotherapeutic drug selection. This can be achieved by identifying the underlying determinant to drug response, which may significantly reduce the failure rate of cancer chemotherapy.

Recently developed technology for assessment of the human genome enables the identification of genetic variation(s)

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responsible for pathological events that have been caused, for example, by loss-of-heterozygocity (LOH) or alterations in gene copy number.[2–4] Application of this technology has already allowed us to observe characteristic expression of gene clusters associated with good and poor tumour prognosis.[5–7] The gene copy number may determine the 'strength of phenotype', in that more copies may provide an increase in overall gene product activity. Those genes encoding drug activating or deactivating enzymes are of particular interest.

We used the data mining procedure employing the cumulative hypergeometric probability to identify regions of the genome with highly enriched copy numbers. The copy numbers were then correlated with cancer cell line sensitivity to 5-FU. We validated the observed relationships using genetic material derived from the xenografts of human cancers with well established *in vivo*sensitivity levels to 5-FU.

2. Materials and methods

2.1. Data mining source, statistical analysis and software

The National Cancer Institute database (NCI; http://dtp.nci.nih.gov) containing data from 58 NCI60 cell lines was used as the main source of gene copy number,[8] 5-FU and tegafur cytotoxicity data, and karyotype information.[9] Downloaded data were processed and then loaded into GeneSpring™ software, version 7.2 (SiliconGenetics, Redwood, CA). All markers were categorised according to their defined physical position and cytogenetic band on the chromosomes.

To determine those discriminating markers that influence 5-FU sensitivity, the copy number profiles of the drug-sensitive and drug-resistant groups of cancer cell lines were compared using unpaired t-tests (with Welch's correction for unequal variances) calculating the statistical significance of non-random representation at each cytogenetic band. This overlapping *P*-value was calculated using hypergeometric probability according to the following equation:

$$p = \frac{\sum_{i=k}^{n} \binom{m}{i}\binom{u-m}{n-i}}{\binom{u}{n}},$$

where the probability (*P*) of overlap corresponding to *k* or more markers between a marker list of *n* markers is compared against a marker list of *m* markers randomly sampled from a universe of *u* markers.

cDNA microarray data were downloaded from the NCI60 Cancer Microarray Project web site at Stanford University (http://genome-www.stanford.edu/nci60/index.shtml). Fluorescence intensity data were loaded and normalised by 'LOWESS normalization' and 'per gene normalization'. Correlation coefficients were calculated using Spearman Rho and Tukey-Kramer HSD tests with JMP5.0 software (SAS Institute, Tokyo, Japan).

2.2. Human tumour xenografts and examination of antitumour activity

In vivo experiments investigating the sensitivity of human tumour xenografts to orally administered fluorinated pyrimidine drugs at maximum tolerated doses have been described previously.[10] Based on these experiments, 31 human cancer cell line xenografts representing the five main tumour types were tested for sensitivity to the fluorinated pyrimidines UFT, TS-1, capecitabine (synthesised in our laboratory) and 5′-DFUR (Nippon Roche K. K., Tokyo, Japan). The 31 cell lines were breast (MC-5, H-31, MC-2, MX-1, MDA-MB-435SHM, MDA-MD-231), colon (KM12C, HCT-15, KM20C, COL-1, KM12C/FU, CO-3), lung (GT3TKB, LC-11, Lu-99, LX-1, LC-6, Lu-134, Lu-130), pancreas (PAN-3, PAN-4, PAN-12, H-48, MIA-PaCa-2, BxPC-3) and gastric (AZ-521, SC-2, ST-40, 4-1ST, SC-4, OCUM-2MD3). An in-house database was created from these results.

2.3. Genomic DNA preparation and quantitative real-time PCR

Genomic DNA was extracted from 20 to 30 mg xenograft tissue samples using the QIAAMP DNA mini kit (Qiagen Inc., Valencia, CA) according to the manufacturer's instructions. Quantitative real-time PCR was performed to determine *DPD* copy number on a PRISM 7300 sequence detector (Applied Biosystems, Foster City, CA) using a QuantiTect SYBR Green kit (Qiagen) and the following primers: Forward primer (5′-CGGCCCTAGTCTGCCTGTT-3′), reverse primer (5′-GAGTCTGCCAGTGACAAACCCT-3′). We quantified the DNA of each tumour by comparing the target locus to the reference Line-1, a repetitive element for which copy numbers per haploid genome are similar among all normal and neoplastic human cells. The quantification is based on standard curves from a serial dilution of human genomic DNA. The relative target copy number level was also normalised to normal human genomic DNA as calibrator. The copy number change of the target gene relative to the Line-1 and the calibrator were determined by using the formula $(T_{target}/T_{Line\text{-}1})/(C_{target}/C_{Line\text{-}1})$, where T_{target} and $T_{Line\text{-}1}$ are quantities from tumour DNA by using target and Line-1, and C_{target} and $C_{Line\text{-}1}$ are quantities from calibrator by using target and Line-1.[11]

2.4. Quantification of mRNA expression and DPD enzymatic activity

Total RNA was extracted from 20 to 30 mg xenograft tissue samples using the RNeasy kit (Qiagen) according to the manufacturer's instructions. Quantitative real-time RT-PCR was performed on a PRISM 7700 sequence detector (Applied Biosystems) using a TaqMan EZ RT-PCR kit as previously described.[12] Amplification of specific PCR products was detected using dual-fluorescent non-extendable probes labelled with 6-carboxyfluorescein (FAM) at the 5′ end and with 6-carboxytetramethylrhodamine (TAMRA) at the 3′ end. Human gene specific primers and TaqMan probe to amplify *DPD* were 5′-AATGATTCGAAGAGCTTTTGAAGC-3′ (forward primer), 5′-GTTCCCCGGATGATTCTGG-3′ (reverse primer), 5′-TGCCCTCACCAAAACTTTCTCTCTTGATAAGGA-3′ (TaqMan probe), generating a PCR fragment of 108 bp: primers and probe for *ACTB* were 5′-TCACCCACACTGTG CCCATCTACGA-3′ (forward primer), 5′-CAGCGGAACCGCTCATTGCCAATGG-3′ (reverse primer), 5′-ATGCCCTCCCCCATGCCATCCTGCGT-3′ (TaqMan probe), generating a PCR fragment of 295 bp: primers

and probe for *GAPDH* were 5′-GAAGGTGAAGGTCGGAGTC-3′ (forward primer), 5′-GAAGATGGTGATGGGATTTC-3′ (reverse primer), 5′-CAAGCTTCCCGTTCTCAGCC-3′ (TaqMan probe), generating a PCR fragment of 226 bp. DPD mRNA expression level was normalised using the geometric mean of those for *GAPDH* and *ACTB*.

DPD enzymatic activity was measured as previously described.[13] In brief, tumour tissues were sonicated in 4 volumes of homogenisation buffer [20 mM potassium phosphate (pH 8.0) containing 1 mM EDTA and 1 mM β-mercaptoethanol]. Each homogenate was centrifuged at 105,000*g* for 1 h at 4°C, and its supernatant (cytosol) was collected. The enzyme reaction mixture, which contained 10 mM potassium phosphate (pH 8.0), 0.5 mM EDTA, 0.5 mM β-mercaptoethanol, 2 mM dithiothreitol, 5 mM $MgCl_2$, 20 μM [6-^{14}C] 5-FU (American Radiolabeled Chemicals Inc., St. Louis, MO), 100 μM NADPH and 25 μl of the cytosol fraction in a final volume of 50 μl, was incubated at 37 °C for 30 minutes. Upon termination of the reaction, an aliquot (5 μl) of supernatant was applied to thin-layer chromatography (Silica gel 60_{F254}; Merck, Germany) and developed with a mixture of ethanol and 1 M ammonium acetate (5:1, v/v). DPD activity was then determined by measuring the sum of the dihydrofluorouracil and 2-fluoro-β-alanine released from [6-^{14}C] 5-FU.

3. Results

3.1. Data mining in the NCI database

In order to determine the relationship between the cytotoxic effects of 5-FU (NSC19893) and genome-wide DNA copy numbers defined by high-density 125K arrays, the NCI60 panel cell lines were arbitrarily classified as 5-FU-high- and 5-FU-low-sensitive cell lines according to their respective GI50 values. The 5-FU-high-sensitive cell lines were those with GI50 values within the 25th percentile, while the 5-FU-low-sensitive cell lines were above the 75th percentile. The remaining cell lines were classified as intermediate sensitivity.

Welch's *t*-test analysis without any multiple test correction revealed that the copy numbers of 8468 out of 126,000 markers differed significantly ($P < 0.05$) between 5-FU-high- and -low-sensitive cell lines. These 8468 markers, including 6300 coincidentally extracted markers, were then subjected to similarity analysis using hypergeometric probability against the marker lists for each cytogenetic band. The coincidentally extracted markers were expected to be distributed randomly throughout the genome; therefore, by choosing an adequate cutoff value in the similarity analysis, it should be possible to identify statistically significant candidate loci that determine sensitivity to 5-FU cytotoxicity. Eleven of the 862 cytobands tested were identified based on a *P*-value lower than the defined cutoff value of $1 \times 10E{-}40$. These bands are potential candidate loci related to 5-FU cytotoxic effects (Table 1) and contain more than 90 overlapping markers. The average copy number of all markers on 1p21.3 was higher in 5-FU-low-sensitive cell lines than in 5-FU-high-sensitive cell lines. By contrast, the average copy number of markers contained within the remaining 10 cytogenetic bands was higher in 5-FU-high-sensitive cell lines than in 5-FU-low-sensitive cell lines.

3.2. DPD copy number and 5-FU cytotoxicity

As increased resistance to 5-FU is associated with a parallel increase in DNA copy number, it seemed probable that the DNA encoded an enzyme involved in 5-FU catabolism. Based on the findings of recent clinical translational studies,[14,15] dihydropyrimidine dehydrogenase (DPD) was a likely candidate for the rapid and direct disactivation of 5-FU. The *DPD* copy number of cytogenetic band 1p21.3 was thus the first to be investigated.

As shown in Fig. 1, the cytotoxic effects of 5-FU and tegafur were significantly correlated with *DPD* copy number. Although the cytotoxic potency of tegafur is two orders of magnitude weaker than that of 5-FU, the correlation trends are very similar. Further karyotype analysis of chromosomes

Table 1 – Summary of data mining results of the NCI-DTP dataset

Candidate cytobands influencing 5-FU sensitivity (data mining)			125K array data[a] Average copy number		SKY/M-FISH analysis[b] Average copy number	
Chromosome	Cytogenetic band	*P*-value	5-FU S[c]	5-FU R[d]	5-FU S[c]	5-FU R[d]
1	p21.3	2.77E−44	1.69	2.02	2.21	3.13
12	p13.31	1.69E−76	1.95	1.52	2.47	2.51
12	q21.1	1.83E−49	1.99	1.65	2.83	2.82
12	q21.2	8.82E−109	1.98	1.60	2.83	2.82
12	q21.31	4.85E−138	1.98	1.55	2.83	2.82
12	q21.33	1.89E−109	2.00	1.56	2.83	2.82
12	q22	3.50E−99	2.13	1.67	2.78	2.34
12	q23.1	5.81E−125	2.09	1.65	2.78	2.22
13	q13.3	3.43E−44	1.85	1.51	2.77	2.53
13	q14.11	2.93E−65	2.00	1.58	2.89	2.58
16	q23.1	2.18E−68	2.04	1.72	2.30	2.55

a Affymetrix 125K SNP array data.[8]
b Estimated chromosomal band copy number from spectral karyotyping.[9]
c 5-FU sensitive cell lines.
d 5-FU resistant cell lines.

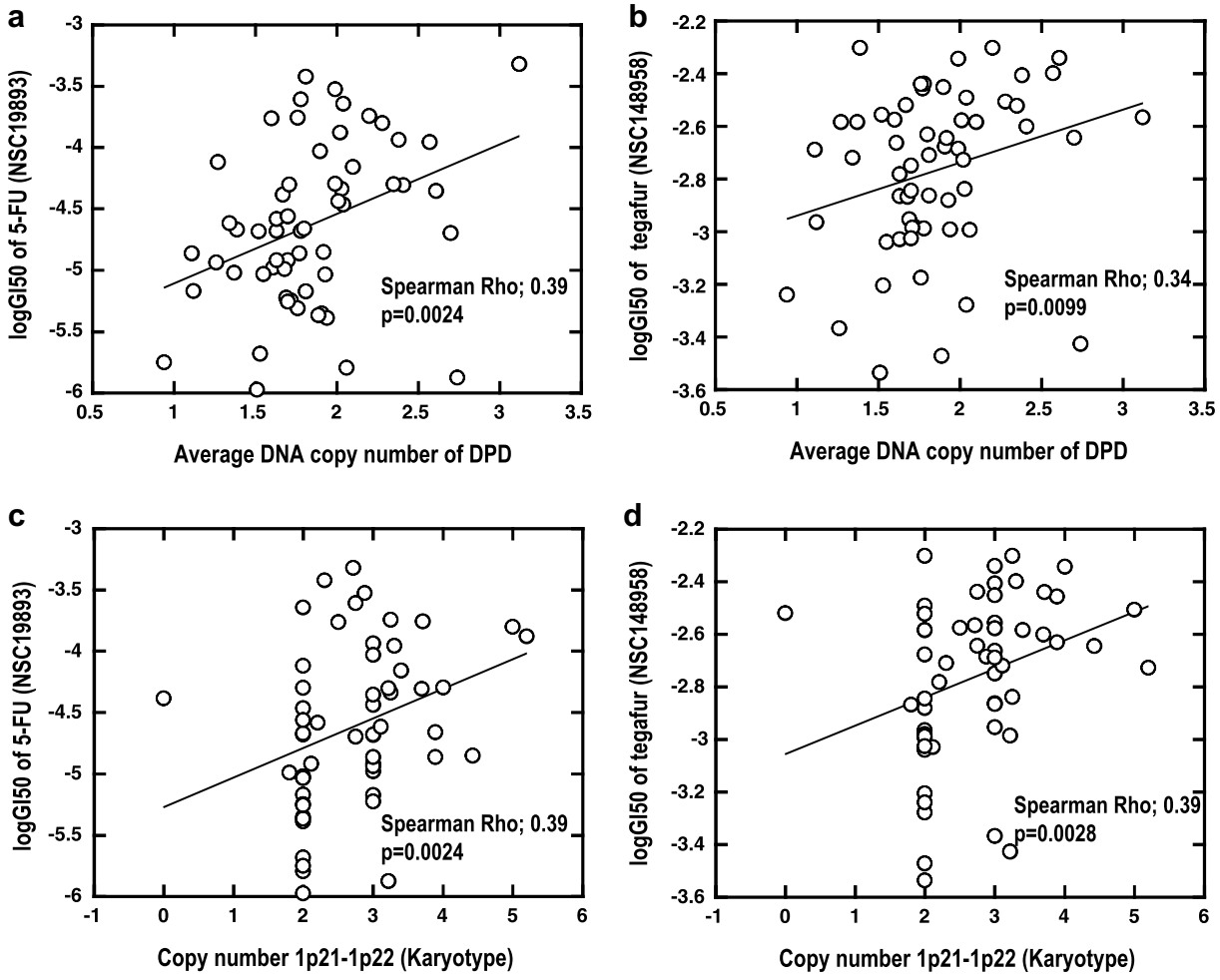

Fig. 1 – The relationship between the cytotoxic effects of 5-FU (NSC19893) or tegafur (NSC148958) and gene copy number in 58 NCI60 panel cell lines as determined by two discrete methods. (a) Plot of log(GI50) of 5-FU or (b) tegafur against the average *DPD* copy number calculated from the 125K-SNPs array data set. (c) Plot of log(GI50) of 5-FU or (d) tegafur against the 1p21-22 copy number from karyotype analysis.

1p21 or 1p22 confirmed the correlation between copy number and cytotoxicity of the drugs; however, this primary indication is complicated by the presence of other compensative mechanisms involved in neutralisation of 5-FU. To confirm the strength of this correlation, the average *DPD* copy numbers were compared with the level of DPD mRNA expression as determined by cDNA microarray analysis within the NCI60 project. As shown in Fig. 2, a significant relationship between DNA copy number and DPD mRNA expression was observed ($R = 0.41$, $P = 0.0014$). Moreover, a significant increase in DPD mRNA expression was observed in cell lines possessing more than two copies of *DPD*.

3.3. *DPD copy number analysis of human tumour xenografts*

To confirm whether the correlation between the *DPD* copy number on chromosome 1p21.3 and 5-FU cytotoxicity is a general phenomenon, we used SYBR-green real time PCR to estimate the *DPD* copy number in 31 human tumour xenografts with a known response to 5-FU-related compounds.

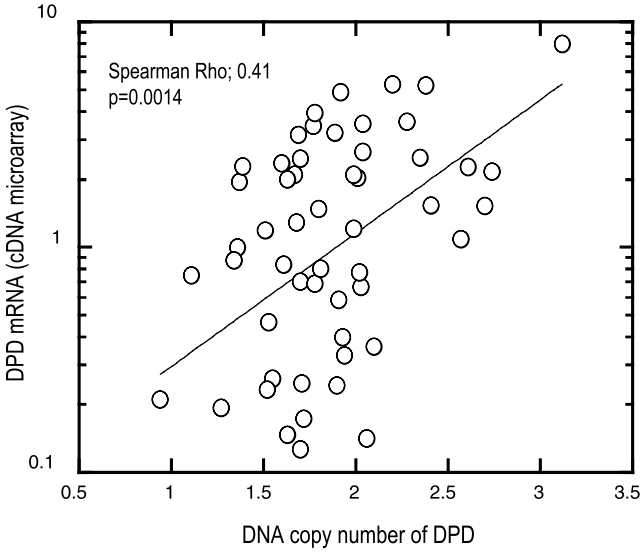

Fig. 2 – Relationship between *DPD* copy number and mRNA expression levels as determined by cDNA microarray in 58 NCI60 panel cell lines.

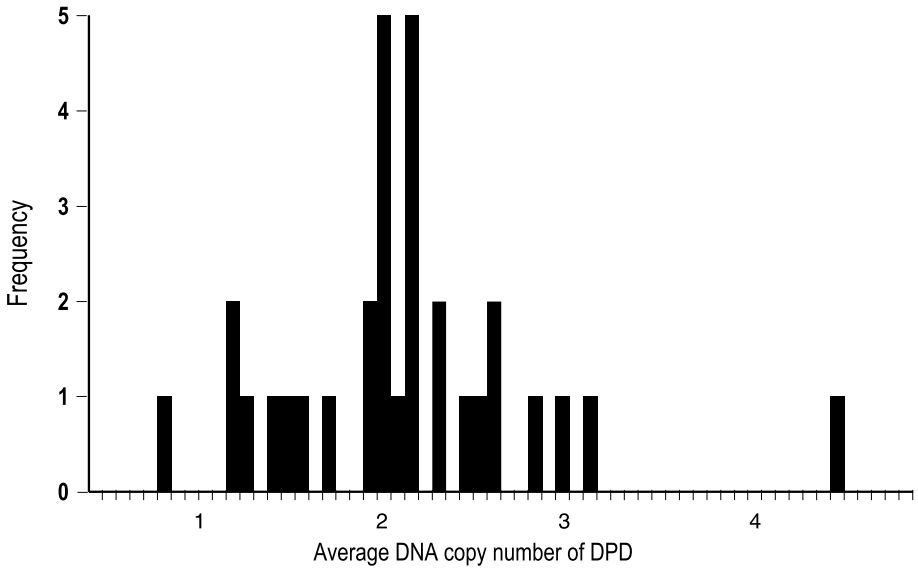

Fig. 3 – Histogram of *DPD* copy number in 31 human tumour xenografts as determined by quantitative real-time PCR.

The panel of human cancer xenografts was unrelated to the cell lines of the NCI60 project, except for KM12C, HCT-15 and MDA-MB231.

Fig. 3 shows that the *DPD* copy number varied from 0.78 to 4.4. Since the expression of DPD mRNA in the NCI60 panel cell lines was significantly increased when present at more than two copies, the fact that 16 of the 31 xenografts had a copy number greater than two suggests that their sensitivity to 5-FU would be low.

3.4. *DPD copy number and fluoropyrimidine anti-tumour activity in human tumour xenografts*

The *in vivo* antitumour activity of UFT, 5′DFUR, capecitabine and TS-1 in nude mice implanted with different human tumour xenografts after standardised treatment is shown as a heat-map in Fig. 4. Based on unsupervised hierarchical clustering, the *DPD* copy number is inversely proportional to the antitumour activity of 5-FU-based drugs. The cancers can

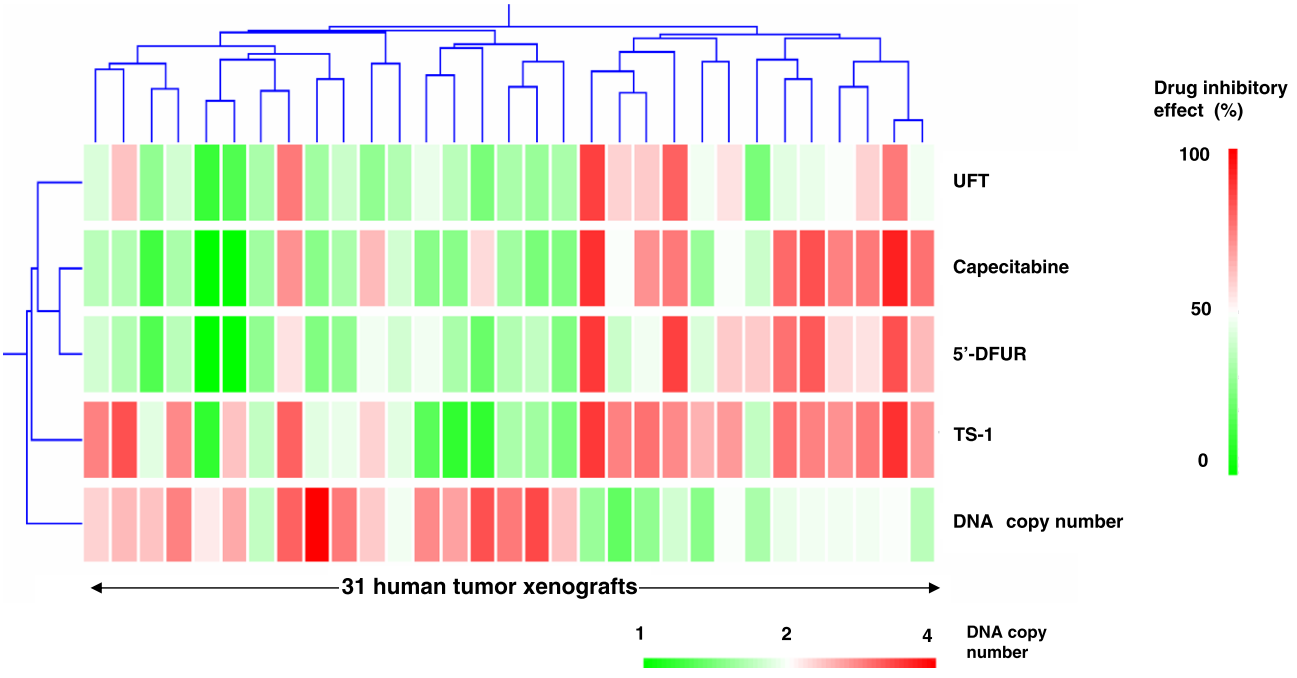

Fig. 4 – Unsupervised hierarchical clustering of 31 human tumour xenografts by *in vivo* susceptibility to 5-FU based drugs and *DPD* copy number.

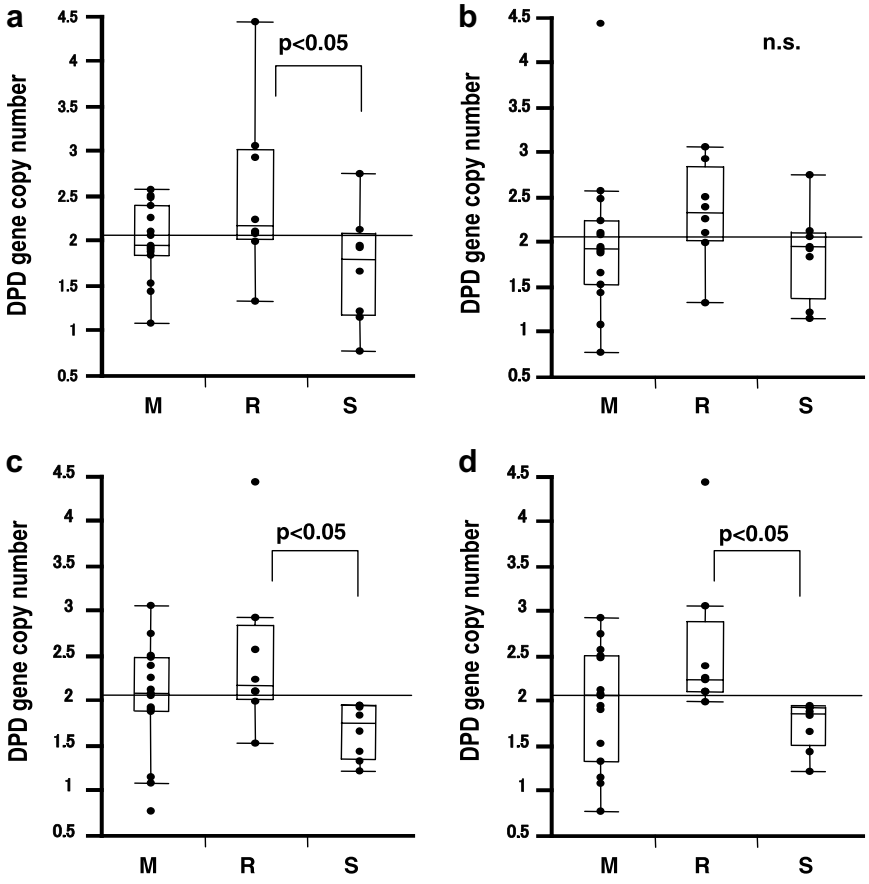

Fig. 5 – Comparison of *DPD* copy number and *in vivo* sensitivity to the 5-FU-based drugs (a) UFT, (b) TS-1, (c) 5′DFUR and (d) capecitabine. Cancers were classified as drug resistant (R), drug sensitive (S), or drug moderate sensitive (M), according to quartile values. The *P*-value of each plot, computed by the Tukey–Kramer HSD test, represents the differences among a set of means that controls the significance level for multiple comparisons. The quantile box plots summarising the distribution of copy number in each group and grand mean lines are shown.

therefore be classified into three groups: drug resistant, drug sensitive and moderately sensitive xenografts according to their quartile values.

The Tukey–Kramer HSD test revealed that the *DPD* copy number differed significantly between drug-resistant and drug-sensitive xenografts following treatment with UFT, 5′DFUR and capecitabine ($P < 0.05$) (Fig. 5). However, the difference in copy number between the two groups of xenografts is not significant following treatment with TS-1.

3.5. *DPD copy number, mRNA expression and enzymatic activity*

A significant correlation was observed between *DPD* copy number and mRNA expression level (Spearman $\rho = 0.36$, $P = 0.046$; Fig. 6). The *DPD* copy number was also positively correlated with DPD enzymatic activity (Spearman $\rho = 0.33$, $P = 0.068$).

4. Discussion

A low response rate of cancers to chemotherapy is often caused by pre-existing natural mechanisms of defence against cytotoxic drugs. This could be due to an increased copy number of the genes encoding drug-deactivation enzymes. To test this hypothesis, we identified chromosome 1p21.3 as a candidate locus that is associated with cancer cell susceptibility to 5-FU. As the gene encoding the enzyme DPD is located in region 1p21.3, we determined the *DPD* copy number in 31 xenografts and showed it to be significantly correlated with *in vivo* sensitivity to 5-FU-based drugs. Interestingly, among the four 5-FU-based drugs tested, TS-1 sensitivity does not correlate with the *DPD* copy number. One may explain this by the fact that TS-1 is a complex drug consisting of tegafur, oxonic acid and CDHP, the latter being a strong inhibitor of DPD activity. Therefore, the presence of a 5-FU-destructive enzyme is neutralised by CDHP, thus minimising DPD-related resistance to 5-FU.

5-FU is converted intracellularly to fluorodeoxyuridine monophosphate, and forms a stable ternary complex with thymidylate synthetase (TS), resulting in the inhibition of an enzyme vital for DNA synthesis. DPD catabolises endogenous pyrimidines and pyrimidine-based antimetabolite drugs such as 5-FU. Many studies have shown that DPD enzymatic activity or tumour expression levels of DPD mRNA are predictive markers for the response to 5-FU-based chemotherapy.[16,17] Salonga et al. reported a significant correlation between

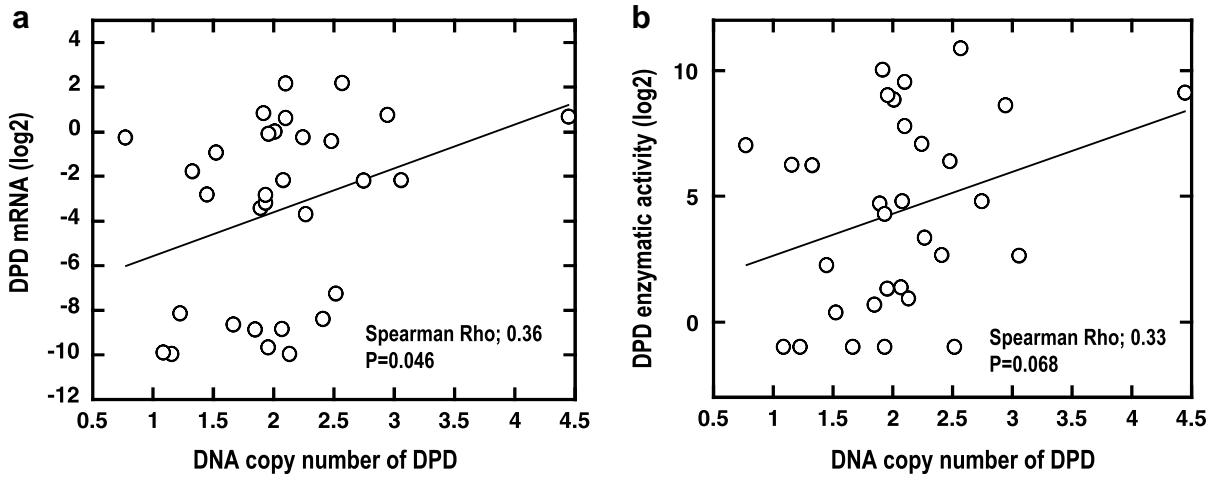

Fig. 6 – DPD mRNA expression (a) and DPD enzymatic activity (b) plotted against *DPD* copy number of 31 human tumour xenografts. Enzymatic activity is expressed as pmol/min/mg protein.

DPD expression levels in colorectal tumours, tumour responsiveness to 5-FU treatment and, most importantly, patient survival.[18] They determined a cutoff value of DPD expression level, above which no tumours responded to 5-FU treatment.

However, this optimal cutoff value may vary among clinical institutions due to mRNA instability, variation in PCR efficacy and other technical differences. Here, we showed that the *DPD* copy number in NCI60 cell lines and in 31 human tumour xenografts significantly correlated with DPD mRNA expression. Therefore, stable DNA, rather than fragile RNA, might be a more useful marker for predicting clinical outcome; indeed, we propose a stable cutoff value of two gene copies per nucleus.

Ota et al. reported that chromosome 1 aberrations did not correlate with DPD expression in micro tissue specimens of breast cancer.[19] This might be caused by a high discrepancy in the number of cases of disomy ($n = 6$) and polysomy ($n = 38$). Alternatively, it could be a result of the large distance between the centromeric chromosome 1 fluorescent *in situ* hybridisation (FISH) probe and the *DPD* gene on chromosome 1p21.3.

We believe that the wide application of DNA-based probes in analyses such as FISH and comparative genomic hybridisation (CGH) will provide more precise information on the sensitivity of a particular cancer to 5-FU treatment. Although there are already a wide range of valuable anticancer drugs, they are not always effective for particular cancer types or certain populations of cancer patients. The unusual responses of cancers to chemotherapy provide information on the feed-back mechanism(s) of cancer survival after exposure to harsh conditions such as those induced by anticancer agents. For instance, the overexpression of cell membrane pumps,[20] modification of cell receptors in the withdrawal syndrome of prostate cancers,[21] and overexpression of drug-metabolising enzymes[22] play a crucial role in neutralising the effects of anticancer drugs in cancer cells. In the case of 5-FU related compounds, other molecular targets must be taken into account such as TS and thymidine phosphorylase. Overexpression of TS is associated with a poor response rate,[23] while thymidine phosphorylase may be essential for the activation of 5-FU,[24] or may be responsible for the rapid deactivation of 5-FU drugs, resulting in a weak anticancer activity.

The correlation between *DPD* copy number and sensitivity to 5-FU drugs is a first step towards the improvement of chemotherapy. It is now important to expand our studies to other genes associated with 5-FU sensitivity to precisely discriminate against non-responding cancers. Although a recent study on the correlation between gene copy number and drug sensitivity in the NCI-60 cell panel did not identify 5-FU as a drug associated with copy number, this could be a result of the limited number of unique loci tested.[25] However, the final results of both this and the current study can be considered complementary.

Considering recent trends in the field of drug development and efforts made by the FDA in providing safe and effective treatment (The Critical Path Initiative: http://www.fda.gov/oc/initiatives/criticalpath/), the introduction of a specific test such as the *DPD* copy number may meet social expectations. The analysis is less prone to error than other techniques used to date, and is affordable under health insurance systems.

Conflict of interest statement

None declared.

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REFERENCES

1. van Triest B, van Groeningen CJ, Pinedo HM. Current chemotherapeutic possibilities in the treatment of colorectal cancer. *Eur J Cancer A* 1995;**31**:1193–7.

2. Callagy G, Pharoah P, Chin SF, et al. Identification and validation of prognostic markers in breast cancer with the complementary use of array-CGH and tissue microarrays. *J Pathol* 2005;**205**:388–96.
3. Weiss MM, Kuipers EJ, Postma C, et al. Genomic alterations in primary gastric adenocarcinomas correlate with clinicopathological characteristics and survival. *Cell Oncol* 2004;**26**:307–17.
4. Rubio-Moscardo F, Climent J, Siebert R, et al. Mantle-cell lymphoma genotypes identified with CGH to BAC microarrays define a leukemic subgroup of disease and predict patient outcome. *Blood* 2005;**105**:4445–54.
5. Sotiriou C, Wirapati P, Loi S, et al. Gene expression profiling in breast cancer: understanding the molecular basis of histologic grade to improve prognosis. *J Natl Cancer Inst* 2006;**98**:262–72.
6. van de Vijver M. Gene-expression profiling and the future of adjuvant therapy. *Oncologist* 2005;**10**(Suppl. 2):30–4.
7. Barrier A, Boelle PY, Lemoine A, et al. Gene expression profiling of nonneoplastic mucosa may predict clinical outcome of colon cancer patients. *Dis Colon Rectum* 2005;**48**:2238–48.
8. Garraway LA, Widlund HR, Rubin MA, et al. Integrative genomic analyses identify MITF as a lineage survival oncogene amplified in malignant melanoma. *Nature* 2005;**436**:117–22.
9. Roschke AV, Tonon G, Gehlhaus KS, et al. Karyotypic complexity of the NCI-60 drug-screening panel. *Cancer Res* 2003;**63**:8634–47.
10. Ooyama A, Takechi T, Toda E, et al. Gene expression analysis using human cancer xenografts to identify novel predictive marker genes for the efficacy of 5-fluorouracil-based drugs. *Cancer Sci* 2006;**97**:510–22.
11. Zhao X, Li C, Paez JG, et al. An integrated view of copy number and allelic alterations in the cancer genome using single nucleotide polymorphism arrays. *Cancer Res* 2004;**64**:3060–71.
12. Yoshinare K, Kubota T, Watanabe M, et al. Gene expression in colorectal cancer and in vitro chemosensitivity to 5-fluorouracil: a study of 88 surgical specimens. *Cancer Sci* 2003;**94**:633–8.
13. Takechi T, Fujioka A, Matsushima E, Fukushima M. Enhancement of the antitumour activity of 5-fluorouracil (5-FU) by inhibiting dihydropyrimidine dehydrogenase activity (DPD) using 5-chloro-2,4-dihydroxypyridine (CDHP) in human tumour cells. *Eur J Cancer* 2002;**38**:1271–7.
14. Kidd EA, Yu J, Li X, et al. Variance in the expression of 5-fluorouracil pathway genes in colorectal cancer. *Clin Cancer Res* 2005;**11**:2612–9.
15. Smorenburg CH, Peters GJ, van Groeningen CJ, et al. Phase II study of tailored chemotherapy for advanced colorectal cancer with either 5-fluouracil and leucovorin or oxaliplatin and irinotecan based on the expression of thymidylate synthase and dihydropryimidine dehydrogenase. *Ann Oncol* 2006;**17**:35–42.
16. Grem JL, Danenberg KD, Behan K, et al. Thymidine kinase, thymidylate synthase, and dihydropyrimidine dehydrogenase profiles of cell lines of the National Cancer Institute's Anticancer Drug Screen. *Clin Cancer Res* 2001;**7**:999–1009.
17. Etienne MC, Cheradame S, Fischel JL, et al. Response to fluorouracil therapy in cancer patients: the role of tumoral dihydropyrimidine dehydrogenase activity. *J Clin Oncol* 1995;**13**:1663–70.
18. Salonga D, Danenberg KD, Johnson M, et al. Colorectal tumors responding to 5-fluorouracil have low gene expression levels of dihydropyrimidine dehydrogenase, thymidylate synthase, and thymidine phosphorylase. *Clin Cancer Res* 2000;**6**:1322–7.
19. Ota D, Kusama M, Kaise H, et al. Evaluation of sensitivity to 5-FU on the basis of thymidylate synthase (TS)/ dihydropyrimidine dehydrogenase (DPD) activity and chromosomal analysis in micro tissue specimens of breast cancer. *Breast Cancer* 2004;**11**:356–66.
20. Gottesman MM, Fojo T, Bates SE. Multidrug resistance in cancer: role of ATP-dependent transporters. *Nat Rev Cancer* 2002;**2**:48–58.
21. Bohl CE, Gao W, Miller DD, et al. Structural basis for antagonism and resistance of bicalutamide in prostate cancer. *Proc Natl Acad Sci USA* 2005;**102**:6201–6.
22. Hamilton M, Wolf JL, Zborowski D. Tarceva™ (erlotinib) exposure/effects (EE) analysis from a Phase III study in advanced NSCLC: effect of smoking on the PK of erlotinib. AACR Annual Meeting, Anaheim, Orange County, CA, USA; 2005.
23. Johnston PG, Lenz HJ, Leichman CG, et al. Thymidylate synthase gene and protein expression correlate and are associated with response to 5-fluorouracil in human colorectal and gastric tumors. *Cancer Res* 1995;**55**: 1407–1412.
24. de Bruin M, van Capel T, Van der Born K, et al. Role of platelet-derived endothelial cell growth factor/thymidine phosphorylase in fluoropyrimidine sensitivity. *Br J Cancer* 2003;**88**:957–64.
25. Bussey KJ, Chin K, Lababidi S, et al. Integrating data on DNA copy number with gene expression levels and drug sensitivities in the NCI-60 cell line panel. *Mol Cancer Ther* 2006;**5**:853–67.