Antimetabolite drugs work by inhibiting essential biosynthetic processes, or by being incorporated into macromolecules, such as DNA and RNA, and inhibiting their normal function. The fluoropyrimidine 5-fluorouracil (5-FU) does both. FLUOROPYRIMIDINES were developed in the 1950s following the observation that rat hepatomas used the pyrimidine uracil — one of the four bases found in RNA — more rapidly than normal tissues, indicating that uracil metabolism was a potential target for antimetabolite chemotherapy1. The mechanism of cytotoxicity of 5-FU has been ascribed to the misincorporation of fluoronucleotides into RNA and DNA and to the inhibition of the nucleotide synthetic enzyme thymidylate synthase (TS).

5-FU is widely used in the treatment of a range of cancers, including colorectal and breast cancers, and cancers of the aerodigestive tract. Although 5-FU in combination with other chemotherapeutic agents improves response rates and survival in breast and head and neck cancers, it is in colorectal cancer that 5-FU has had the greatest impact. 5-FU-based chemotherapy improves overall and disease-free survival of patients with resected stage III colorectal cancer2. Nonetheless, response rates for 5-FU-based chemotherapy as a first-line treatment for advanced colorectal cancer are only 10–15% (REF. 3). The combination of 5-FU with newer chemotherapies such as irinotecan and oxaliplatin has improved the response rates for advanced colorectal cancer to 40–50% (REFS 4,5). However, despite these improvements, new therapeutic strategies are urgently needed.

Understanding the mechanisms by which 5-FU causes cell death and by which tumours become resistant to 5-FU is an essential step towards predicting or overcoming that resistance. So, what do we know about the mechanism of action of 5-FU and what strategies have been used to enhance its activity? DNA MICROARRAY technology has the potential to identify novel genes that are involved in mediating resistance to 5-FU. Such genes might prove to be therapeutically valuable as new targets for chemotherapy, or as predictive biomarkers of 5-FU chemosensitivity and/or provide new molecular targets that overcome drug resistance. How have pre-clinical studies impacted on the clinical use of 5-FU and how might DNA microarray profiling affect its future clinical application?

5-FU is an analogue of uracil with a fluorine atom at the C-5 position in place of hydrogen (FIG. 1). It rapidly enters the cell using the same facilitated transport mechanism as uracil6. 5-FU is converted intracellularly to several active metabolites: fluorodeoxyuridine monophosphate (FdUMP), fluorodeoxyuridine triphosphate (FdUTP) and fluorouridine triphosphate (FUTP) (FIG. 1) — these active metabolites disrupt RNA synthesis and the action of TS. The rate-limiting enzyme in 5-FU catabolism is dihydropyrimidine dehydrogenase (DPD), which converts 5-FU to dihydrofluorouracil (DHFU). More than 80% of administered 5-FU is normally catabolized primarily in the liver, where DPD is abundantly expressed.
TS inhibition. TS catalyses the reductive methylation of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP), with the reduced folate, 5,10-methylenetetrahydrofolate (CH2THF) as the methyl donor (FIG. 2). This reaction provides the sole de novo source of thymidylate, which is necessary for DNA replication and repair. The 36-kDa TS protein functions as a dimer, both subunits of which contain a nucleotide-binding site and a binding site for CH2THF. The 5-FU metabolite FdUMP binds to the nucleotide-binding site of TS, forming a stable ternary complex with the enzyme and CH2THF, thereby blocking binding of the normal substrate dUMP and inhibiting dTMP synthesis1 (FIG. 2).

The exact molecular mechanisms that mediate events downstream of TS inhibition have not been fully elucidated. Depletion of dTMP results in subsequent depletion of deoxythymidine triphosphate (dTTP), which induces perturbations in the levels of the other deoxynucleotides (dATP, dGTP and dCTP) through various feedback mechanisms20. Deoxynucleotide pool imbalances (in particular, the dATP/dTTP ratio) are thought to severely disrupt DNA synthesis and repair, resulting in lethal DNA damage21,22 (FIG. 2). In addition, TS inhibition results in accumulation of dUMP, which might subsequently lead to increased levels of deoxyuridine triphosphate (dUTP)13,14. Both dUTP and the 5-FU metabolite FdUTP can be misincorporated into DNA. Repair of uracil and 5-FU-containing DNA by the nucleotide excision repair enzyme uracil-DNA-glycosylase (UDG)15 is futile in the presence of high (F)dUTP/dTTP ratios and only results in further false-nucleotide incorporation. These futile cycles of misincorporation, excision and repair eventually lead to DNA strand breaks and cell death. DNA damage due to dUTP misincorporation is highly dependent on the levels of the pyrophosphatase dUTPase, which limits intracellular accumulation of dUTP16,17 (FIG. 2). Thymidylate can be salvaged from thymidine through the action of thymidine kinase, thereby alleviating the effects of TS deficiency (FIG. 2). This salvage pathway represents a potential mechanism of resistance to 5-FU18.

RNA misincorporation. The 5-FU metabolite FUTP is extensively incorporated into RNA, disrupting normal RNA processing and function. Significant correlations between 5-FU misincorporation into RNA and loss of clonogenic potential have been shown in human colon and breast cancer cell lines21,22. 5-FU misincorporation can result in toxicity to RNA at several levels. It not only inhibits the processing of pre-mRNA into mature rRNA, tRNA and snRNA, but also disrupts post-transcriptional modification of pre-mRNA and the assembly and activity of snRNA/protein complexes, thereby inhibiting splicing of pre-mRNA23,24. In addition, rRNA, tRNA and snRNA all contain the modified base pseudouridine, and 5-FU has been shown to inhibit the post-transcriptional conversion of uridine to pseudouridine in these RNA species25. Polyadenylation of pre-mRNA is inhibited at relatively low 5-FU concentrations26. These in vitro studies indicate that 5-FU misincorporation can potentially...
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**Figure 2 | Mechanism of thymidylate synthase inhibition by 5-fluorouracil.** Thymidylate synthase (TS) catalyses the conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP) with 5,10-methylene tetrahydrofolate (CH₂THF) as the methyl donor. The 5-fluorouracil (5-FU) active metabolite fluorodeoxyuridine monophosphate (FdUMP) binds to the nucleotide-binding site of TS and forms a stable ternary complex with TS and CH₂THF, blocking access of dUMP to the nucleotide-binding site and inhibiting dTMP synthesis. This results in deoxyuridine triphosphate (dUTP) pool imbalances and increased levels of deoxyuridine triphosphate (dUTP), both of which cause DNA damage. The extent of DNA damage caused by dUTP is dependent on the levels of the pyrophosphatase dUTPase and uracil-DNA glycosylase (UDG). dTMP can be salvaged from thymidine through the action of thymidine kinase (TK).

Disrupt many aspects of RNA processing, leading to profound effects on cellular metabolism and viability.

**Modulation of 5-FU**

5-FU has been used for more than 40 years in the treatment of colorectal cancer. 5-FU is given intravenously and has been used in a variety of different schedules to determine the optimum dose and mode of administration. The overall response rate for 5-FU as a single agent in advanced colorectal cancer is quite limited (approximately 10–15%); however, over the past 20 years, Important modulation strategies have been developed to increase the anticancer activity of 5-FU and to overcome clinical resistance. As a result, 5-FU has remained the main agent for the treatment of both advanced and early-stage colorectal cancer. Strategies that have been explored to modulate the anticancer activity of 5-FU include decreasing 5-FU degradation, increasing 5-FU activation and increasing the TS binding activity of FdUMP (FIG. 3). Some of these strategies are discussed below.

**Leucovorin.** High intracellular levels of the reduced folate CH₂THF are necessary for optimal binding of FdUMP to TS. Leucovorin (LV, 5'-formyltetrahydrofolate) has been used to expand the intracellular concentration of CH₂THF and has been shown to increase the in vitro and in vivo toxicity of 5-FU in many cancer cell lines. LV enters the cell via the reduced folate carrier and is anabolized to CH₂THF, which is then polyglutamated by polyglutamate synthetase. Polyglutamation not only increases the cellular retention of CH₂THF, but also enhances the stabilization of its ternary complex with TS and FdUMP. In cell-free systems, the pentaglutamate form was found to be 40-fold more potent in promoting ternary complex formation than the monoglutamate. The advanced colorectal cancer meta-analysis project (ACCM P) showed that 5-FU/LV generated significantly superior response rates compared with bolus single-agent 5-FU (23% versus 11%); however, this did not result in improved overall survival.

**Inhibitors of dihydropyrimidine dehydrogenase.** 5-FU shows poor bioavailability due to its rapid degradation to DHFU by DPD. Several strategies have been developed to inhibit DPD-mediated degradation of 5-FU. The UFT (uracil/Florafur) formulation uses a 4:1 combination of uracil with the 5-FU pro-drug Florafur, which improves 5-FU bioavailability by saturating DPD with its natural substrate. Douillard and colleagues reported that UFT/LV produced equivalent response rates to 5-FU/LV and was a safer, more convenient treatment. In addition, DPD inhibitors, such as eniluracil and 5-chlorodihydropyrimidine (CDHP), have been investigated. An interesting study by Spector et al. found that eniluracil improved the tumour response rate to 5-FU from 13% to 94% in a rat model. Furthermore, they showed that co-administration of DHFU with 5-FU and eniluracil reduced the response rate to 38%. These findings indicate that 5-FU metabolites might interfere with the antitumour efficacy of 5-FU and provide a further rationale for designing formulations that inhibit DPD.

Another approach has been to design 5-FU prodrugs that avoid DPD-mediated degradation in the liver. Capecitabine is an oral fluoropyrimidine that is absorbed unchanged through the gastrointestinal wall and is converted to 5’-deoxy-5-fluourouridine (5’DFUR) in the liver by the sequential action of carboxylesterase and cytidine deaminase (FIG. 3). 5’DFUR is then converted to 5-FU by thymidine phosphorylase (TP) and/or uridine phosphorylase (UP), both of which have been reported to be significantly more active in tumour tissue than in normal tissue. This might account for the observed tumour-selective activation of capecitabine in 5-FU. In clinical trials, capecitabine showed a significantly higher response rate than 5-FU/LV (24.8% versus 15.5%), although time to disease progression and survival were similar for the two treatment arms. Furthermore, the toxicity profile of capcetabine was more favourable with fewer treatment-related serious adverse events and hospitalizations.

**Methotrexate.** Methotrexate (MTX) is an antifolate inhibitor of dihydrofolate reductase (DHFR), which catalyses the conversion of dihydrofolate (DHF) to tetrahydrofolate (THF). THF is required for purine biosynthesis and, as the precursor of CH₂THF, is also necessary for dTMP synthesis. MTX inhibits both purine and thymidine biosynthesis. In vitro and in vivo studies have shown that MTX can synergize with 5-FU when administered before 5-FU. Inhibition of purine biosynthesis by MTX increases the levels of phosphoribosylpyrophosphate (PRPP), which is the cofactor required for the conversion of 5-FU to fluorouridine monophosphate (FUMP) by orotate phosphoribosyltransferase (OPRT; FIG. 1). So, the increased PRPP levels induced by MTX would promote conversion of 5-FU to
FUMP is converted to 5-FU reported to enhance thymidine phosphorylase (TP) activity, abrogate acute TS induction caused by activation by increasing phosphoribosyl pyrophosphate (PRPP) levels. Interferons (IFNs) have been uracil inhibit DPD-mediated degradation of 5-FU. Methotrexate (MTX) is thought to increase 5-FU thymidylate synthase (TS) inhibition by fluorodeoxyuridine monophosphate (FdUMP). Eniluracil and NATURE REVIEWS be due to random mutations.

Determination of variant DNA sequences in a population at frequencies that are too high to occurrence of variant DNA damage. RNA damage. FUTP is converted to FUMP and transport into the liver by the sequential action of carboxylesterase and cytidine deaminase.

Figure 3 | Modulation of 5-fluorouracil activity. Summary of some of the strategies that have been investigated for increasing the antitumor activity of 5-fluorouracil (5-FU). Leucovorin (LV) increases the intracellular pool of 5,10-methylene tetrahydrofolate (CH2THF), thereby enhancing thymidylate synthase (TS) inhibition by fluorodeoxyuridine monophosphate (FdUMP). Eniluracil and uracil inhibit DPD-mediated degradation of 5-FU. Methotrexate (MTX) is thought to increase 5-FU activation by increasing phosphoribosyl pyrophosphate (PRPP) levels. Interferons (IFNs) have been reported to enhance thymidine phosphorylase (TP) activity, abrogate acute TS induction caused by 5-FU treatment and enhance 5-FU-mediated DNA damage. Capecitabine is a 5-FU pro-drug that is converted to 5′-deoxy-5-fluorouridine (5′DFUR) in the liver by the sequential action of carboxylesterase and cytidine deaminase. 5′DFUR is converted to 5-FU by TP.

Thymidylate synthase. Preclinical studies have demonstrated that TS expression is a key determinant of 5-FU sensitivity. Gene amplification of TS with consequent increases in TS mRNA and protein has been observed in cell lines that are resistant to 5-FU and fluorodeoxyuridine (FUDR). Multiple clinical investigations have measured TS expression by immunohistochemistry and reverse-transcription PCR (RT-PCR) and have shown an improved response to 5-FU-based therapy in patients with low tumoral TS expression. More recently, genotyping of the TS promoter has been reported to divide colorectal cancer patients into those who receive a survival benefit from 5-FU-based chemotherapy and those who do not. The TS gene promoter is polymorphic and usually has either two (TSER*2) or three (TSER*3) 28-base-pair tandem-repeat sequences. Preliminary studies indicate that TSER*3/TSER*3 homozygous patients are less likely to respond to 5-FU-based chemotherapy than TSER*2/TSER*2 homozygous and TSER*2/TSER*3 heterozygous patients. In vitro studies have shown that TS promoters with the TSER*3 sequence generate nearly threefold higher mRNA than those with the TSER*2 sequence. Therefore, the lack of response to 5-FU in TSER*3/TSER*3 patients might be due to higher levels of TS expression in the tumours of these patients compared with patients with the TSER*2/TSER*2 and TSER*2/TSER*3 genotypes. If so, these genotyping studies correlate well with the biochemical, immunohistochemical and RT-PCR-based studies, which have consistently shown that high TS expression predicts a poor acute response to 5-FU-based chemotherapy.

Thyroid kinase inhibitor. Preclinical studies have demonstrated that treatment with 5-FU has been shown to acutely induce TS expression in both cell lines and tumours. This induction seems to be due to inhibition of a negative-feedback mechanism in which ligand-free TS protein binds to and inhibits the translation of TS mRNA. When stably bound by FdUMP, TS is no longer able to bind to its mRNA and suppress its own translation, resulting in increased protein expression.

Determinants of response to 5-FU Another emerging field of clinical investigation is the characterization of the biological factors that correlate with response to 5-FU-based chemotherapy. This approach will have an important role in defining those patients who are most likely to benefit from 5-FU-based chemotherapy in the future.
This constitutes a potential mechanism of resistance, as the acute increase in TS protein levels would facilitate recovery of enzymatic activity.

**Thymidine phosphorylase.** Thymidine phosphorylase (TP) reversibly converts 5-FU to fluorodeoxyuridine (FUDR), which can then be converted to the active metabolite FdUMP (Fig. 1). Characterization of the role of TP in modulating 5-FU responsiveness has been confusing due to contradictory preclinical and clinical data. TP overexpression in cell culture and xenograft models has been shown to increase sensitivity to 5-FU, presumably due to enhanced formation of FdUMP \(^{74}\). However, retrospective analysis of TP mRNA expression in 38 colorectal tumours indicated that tumours with high TP expression were actually less likely to respond to 5-FU \(^{75}\). These contradictory findings might be explained by the fact that TP is also an angiogenic endothelial-cell growth factor and high TP expression in colorectal cancer has been correlated with worse prognosis \(^{76}\). It is possible that high TP expression might be a marker for a more invasive and aggressive tumour phenotype that is less responsive to chemotherapy. However, as tumour cells do not benefit from increased angiogenic potential in tissue culture, TP-mediated activation of 5-FU might predominate in this setting.

**Dihydropyrimidine dehydrogenase.** Patients who are deficient in DPD experience profound systemic toxicity in response to 5-FU \(^{77}\). The cause of this toxicity is prolonged exposure to 5-FU due to decreased drug catabolism. Recent genetic studies have started to define the mutations in the DPD gene that are responsible for the DPD-deficient phenotype \(^{78}\). In vitro studies have also shown that DPD overexpression in cancer cell lines confers resistance to 5-FU \(^{79}\). Furthermore, high levels of DPD mRNA expression in colorectal tumours have been shown to correlate with resistance to 5-FU \(^{80}\). These findings presumably reflect higher DPD-mediated degradation of 5-FU in these tumours. Importantly, the study by Salonga et al. \(^{80}\) also indicated that DPD, TS and TP are each independent predictive markers of 5-FU response and that measurement of all three markers markedly enhanced the ability to predict tumour response to 5-FU-based chemotherapy.

**p53.** The tumour suppressor p53 maintains DNA integrity by transcriptionally activating genes such as CDKN1A and GADD45α, the products of which induce cell-cycle arrest in response to DNA damage \(^{81},^{82}\). However, depending on the cellular context and the nature of the DNA damage, p53 can trigger elimination of the damaged cells by promoting apoptosis through the induction of pro-apoptotic genes, such as *FAS* (*CD95/APO1*) and *BAX*, and the down-regulation of anti-apoptotic *BCL2* (*BCL2*) (Ref. 83). In vitro studies have reported that loss of p53 function reduces cellular sensitivity to 5-FU \(^{84},^{85}\). It has been shown that disrupting both alleles of TP53 or *BAX* in a colon cancer cell line made the cells strikingly resistant to apoptosis induced by 5-FU compared with the parental line \(^{86},^{87}\). We have found that p53 stabilization in response to TS inhibition by the TS-targeted antifolate raltitrexed was abrogated in breast cancer cells by increased TS expression, whereas 5-FU-mediated induction of p53 was insensitive to increased TS expression \(^{88}\). However, p53 stabilization in response to 5-FU was reduced following co-incubation with uridine, which competes with 5-FU for incorporation into RNA. This indicates that 5-FU-mediated stabilization of p53 is primarily due to RNA-directed effects in this cell line. So, both TS inhibition and misincorporation of 5-FU metabolites into RNA result in p53 stabilization (Fig. 4). The signalling pathways that link RNA damage or DNA damage following TS inhibition to p53 activation has not been formally assessed, so the mechanism(s) are unclear at present.

A number of clinical studies have found that p53 overexpression — a surrogate marker for TP53 mutations — correlated with resistance to 5-FU \(^{89},^{91}\). Ahnen and colleagues found no survival benefit from adjuvant 5-FU-based chemotherapy for stage III colorectal cancer patients whose tumours overexpressed TP53 (Ref. 91), but other studies have found no such correlation \(^{92}\). Such conflicting findings might, in part, be due to the wide variation in immunohistochemical protocols, and the use of different antibodies to detect p53. An additional complication might be that p53 overexpression does not actually reflect TP53 mutation in 30–40% of cases \(^{93}\). Other techniques, such as DNA sequencing, might provide a more objective assessment of p53 status. At present, despite the in vitro evidence for p53 involvement in downstream signalling in response to 5-FU, the clinical value of p53 as a predictive marker for 5-FU-based chemotherapy remains a matter for debate.
Microsatellite instability (MSI) is caused by failure of the DNA mismatch-repair (MMR) machinery to correct errors that occur during DNA replication. Deficiencies in the DNA MMR machinery are responsible for 10–15% of all colorectal cancers and >90% of cases of hereditary non-polypoid colon cancer (HNPCC)\(^{[4]}\). In HNPCC, MSI is caused by inherited mutations in MMR genes — most frequently, MLH1, MSH2 and MSH6 (Ref. 99) — whereas most sporadic MSI tumours are caused by transcriptional silencing of the MLH1 gene due to promoter methylation\(^{[10]}\). Meyers and colleagues showed that restoration of MLH1 activity in a MMR-deficient colon cancer cell line increased sensitivity to 5-FU\(^{[11]}\), indicating that MMR-deficient cells are less sensitive to 5-FU. However, the MSI phenotype has been associated with excellent survival in patients who receive adjuvant 5-FU-based chemotherapy\(^{[12]}\). These apparently contradictory findings might be due to intrinsic biological differences between MSI-positive and MSI-negative tumours — for example, most MSI-positive tumours have wild-type p53, whereas most MSI-negative tumours have mutated p53 proteins. It might be that increased 5-FU resistance due to MMR deficiency is less important than increased resistance caused by loss of functional p53, with the result that MSI-positive (TP53-wild-type) tumours are actually more responsive than MSI-negative (TP53-mutant) tumours.

Identification of novel 5-FU biomarkers

It is clearly important that the molecular signalling pathways that link the cellular damage caused by 5-FU to downstream target-gene activation are fully elucidated, as these might affect sensitivity to 5-FU. As well as p53, other key transcriptional regulators are likely to modulate the expression of a cassette of downstream target genes in response to 5-FU. DNA microarray technology has the potential to identify such novel target genes.

The introduction of DNA microarray technology has fundamentally altered the way in which we now address basic biomedical questions. This technology facilitates a more complete and inclusive experimental approach whereby alterations in the transcript level of entire genomes can be simultaneously assayed in response to a variety of stimuli. Such 'transcriptional profiling' can provide comparative data on the relative expression level of individual transcripts in response to a defined stimulus, such as 5-FU treatment. Both overexpression and antisense techniques can be used to define the functional relevance of validated target genes in vitro, whereas the clinical relevance of target genes can be assessed by correlating expression at the protein (immunohistochemistry) or mRNA (real-time RT-PCR) level with tumour response and patient survival.

Several studies have examined the potential of DNA microarrays to predict the response of cancer cells to chemotherapies, including 5-FU\(^{[13-17]}\). Scherf et al. correlated gene expression with the activity of 118 drugs in a panel of 60 human cancer cell lines\(^{[18]}\). Grouping the drugs based on their activity in these cell lines indicated that 5-FU clustered with RNA synthesis inhibitors, indicating that disruption of RNA activity was the predominant mechanism of 5-FU action in these cell lines. However, dialysed serum lacking thymidine was not used in these experiments; therefore, it is possible that thymidine in the culture medium may have abrogated the effects of 5-FU-mediated TS inhibition (as mentioned above, thymidylicate can be salvaged from thymidine through the action of thymidine kinase (FIG. 2)). This study indicated a highly significant inverse correlation between DPD mRNA expression and 5-FU response. This is in agreement with previous studies\(^{[19,20]}\) and validates the approach of using microarrays as diagnostic tools to investigate how variations in the transcription of particular genes relate to mechanisms of chemosensitivity.

A recent study by Zembutsu et al. correlated global gene expression in a panel of 85 cancer xenografts with chemosensitivity to nine anticancer agents, including 5-FU\(^{[21]}\). They established an algorithm that calculated a drug sensitivity score based on the expression of a subset of genes that correlated with drug sensitivity. This study consistently revealed an inverse correlation between TS mRNA levels and 5-FU sensitivity. In addition, the expression levels of the mRNAs encoding the multidrug resistance proteins MDR3 and MDR4 were found to significantly correlate with 5-FU sensitivity.

Similarly, DNA microarray profiling has been used to predict survival of oesophageal cancer patients given adjuvant chemotherapy\(^{[22]}\). A subset of 52 genes that correlated with prognosis and possibly with sensitivity to 5-FU and cisplatin were identified. This study established a drug-response score based on the expression of these 52 genes, which the authors concluded had great potential for predicting prognosis.

The studies outlined above used DNA microarrays as a tool for predicting chemosensitivity before treatment. These studies have also identified genes that might prove to be valuable therapeutic targets. We have recently examined changes in global gene expression in response to 5-FU in a breast cancer cell line to identify novel 5-FU-inducible target genes\(^{[23]}\). Five novel genes that were consistently upregulated by 5-FU in a time- and dose-dependent manner encoded the polyamine catabolic enzymes spermine/spermidine acetyltransferase (SSAT), the phospholipid binding protein annexin II, the G-actin-binding protein thymosin-β-10, the heat-shock protein chaperonin-10 and the chloride ion transporter MAT8. To our knowledge, none of these genes had previously been linked to 5-FU response. In addition, expression of each of these genes (in particular, MAT8) was increased in a 5-FU-resistant colon cancer cell line compared with the parental line, indicating that they might be valuable biomarkers of resistance.

Another gene identified and validated from our DNA microarray screen of 5-FU-inducible target genes was FAS\(^{[24]}\). FAS is a member of the tumour-necrosis factor (TNF) receptor superfamily, which, when bound by its cognate ligand FASL, recruits caspase-8 zymogens via the adaptor protein FADD (FAS-associated death domain)\(^{[25]}\). The FASL/FAS/FADD/caspase-8 complex is known as the...
death-inducing signalling complex (DISC). Caspase 8 is activated at the DISC and subsequently initiates a caspase cascade that results in apoptosis. Several studies have indicated that cell death in response to thymidylate depletion occurs via the FAS signalling pathway in some human colon cancer cell lines. Houghton et al. developed a TS-deficient colon cancer cell line, which underwent apoptosis unless exogenous thymidine was supplemented into the culture medium. They found that withdrawal of thymidine from this cell line in the presence of an anti-FAS monoclonal antibody almost completely protected the cells from loss of clonogenicity. Furthermore, the onset of apoptosis following thymidine withdrawal was associated with increased FASL expression. These findings strongly implicate involvement of the FAS death-receptor pathway in mediating apoptosis of these cells following thymidine depletion.

Proper validation of identified targets is crucial in transcriptional profiling studies. Wang et al. examined global gene expression in a panel of cell lines that are resistant to TS inhibitors. They identified YES1 as a gene that was consistently highly expressed in the resistant cell lines compared with the respective parental line. However, YES1 was concluded to have no role in drug resistance and its increased expression was found to be a consequence of its chromosomal location adjacent to TS, which was amplified in each of the resistant cell lines. This study highlighted the importance of careful interpretation of validated target genes identified by DNA microarray profiling.

Future perspectives
Recently, several new drugs have been developed that are active in advanced colorectal cancer. These include oxaliplatin and irinotecan, which act on targets that are distinct from 5-FU. Oxaliplatin is a platinum-based DNA-damaging agent that carries a bulky 1,2-diaminocyclohexane (DACH) ring that has a different spectrum of action and toxicity to cisplatin. Although oxaliplatin is active as a single agent, it is more active when combined with 5-FU/LV — more than doubling the response rate and significantly improving overall survival and disease-free survival. Irinotecan is a topoisomerase I inhibitor and is active in the first- and second-line treatment of advanced colorectal cancer, in which it has shown improved survival and quality of life when compared with best supportive care in patients who have failed 5-FU/LV. In addition, Phase III clinical trials have shown that irinotecan improved response rates and survival when administered as first-line chemotherapy combined with 5-FU/LV compared with 5-FU/LV alone. The success of the 5-FU/LV/oxaliplatin and 5-FU/LV/irinotecan polychemotherapy regimens is likely to change the treatment of not only advanced colorectal cancer, but also earlier-stage colorectal cancer.

The advent of DNA microarray technology has enormous therapeutic implications for cancer therapy. Greater understanding of the molecular determinants of sensitivity to chemotherapeutic drugs will lead to rationally designed treatment combinations, and the identification of new therapeutic targets and biomarkers, and enable the individualization of patient treatment. In the past, attempts to modulate 5-FU cytotoxicity have focused primarily on increasing activation, decreasing degradation and enhancing TS inhibition. Using DNA microarray profiling to identify the downstream signalling pathways that are involved in tumour-cell response to 5-FU will be a key step in the future development of combined chemotherapy regimens that are designed to enhance the cytotoxic activity of the drug. In addition to identifying potential drug interactions, transcriptional profiling will identify many novel therapeutic targets. Potential targets will first have to be evaluated using in vitro transgenic expression and antisense techniques, followed by studies in animal models.

DNA microarray technology could also be used directly as a therapeutic tool. Some of the initial studies outlined in this review have identified subsets of genes, which, when analysed simultaneously by DNA microarray profiling, predict tumour-cell sensitivity to 5-FU. The study by Salonga et al. clearly shows the benefit of combining information from three molecular biomarkers compared with using a single marker. Increasing the number of biomarkers analysed will further increase our ability to predict drug response; therefore, transcriptional profiling has excellent potential as a means of prospectively identifying patients who are most likely to respond to chemotherapy. Tailoring treatment according to the molecular phenotype of tumour and patient will result in increased tumour response rates. In addition, patients will be spared the toxic side effects of treatment from which they are unlikely to benefit. Higher response rates and decreased toxicity would also reduce the costs of patient care, whereas expensive treatments, such as oxaliplatin or irinotecan, could be used in a more targeted manner.

Future studies might further define a set of key marker genes, which could be used for the prospective evaluation of tumour response to 5-FU and other chemotherapeutic agents.


Shows that analysis of three predictive markers (TS, TP and DPD) markedly enhances the ability to predict tumor response to 5-FU-based chemotherapy compared with using a single biomarker.


85. This clinical study found that patients with stage III colorectal cancer whose tumours overexpressed p53 did not benefit from adjuvant 5-FU-based chemotherapy, indicating that tumours with mutant TP53 are less responsive to 5-FU.


93. One of the first studies to use DNA microarray profiling to identify subsets of genes that have expression levels that correlate with drug sensitivity.


Acknowledgements
This work was supported by Cancer Research UK; the Research and Development Office, Department of Health and Social Services, Northern Ireland, and the Ulster Cancer Foundation.

Online links
DATABASES
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