Chemosensitivity testing for gastrointestinal cancer: survival benefit potential and limitations

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Chemosensitivity testing is considered by some to be a useful method for predicting drug sensitivity of tumor tissues after surgery for gastrointestinal cancer. Although survival benefit is not fully established, several chemosensitivity testing methods have been used clinically, both in the selection of adjuvant therapy and in the treatment of metastatic disease. Chemosensitivity testing is used not only for determination of drug resistance, but also for determination of drug sensitivity conferring a potential survival benefit. Previous retrospective correlation studies showed survival of patients treated with a 'tested' drug to be superior to that of patients treated with a standard drug, but the clinical benefit of chemosensitivity testing in comparison to surgical therapy alone or standard chemotherapy has not been documented in a randomized controlled trial. The clinical usefulness of individualized versus standard therapy needs to be determined. Here we discuss the potential survival benefit and current limitations of chemosensitivity testing in patients with gastrointestinal cancer. *Anti-Cancer Drugs* 14:715–723 © 2003 Lippincott Williams & Wilkins.

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Introduction

Chemosensitivity testing is now performed clinically in cancer patients to determine appropriate drugs to be used in treatment. Such testing began with the human tumor clonogenic assay (HTCA) [1], and several chemosensitivity *in vitro* and *in vivo* assays have since been developed aimed at determining tumor responsiveness to a clinically administered drug. Typical clinically applicable chemosensitivity tests include the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2 H-tetrazolium bromide (MTT) assay [2,3], histoculture drug response assay (HDRA) [4–6] and collagen gel-droplet-embedded-culture drug sensitivity test (CD-DST) [7,8]. Although the survival of gastrointestinal cancer has been improved by early detection by mass screening with gastrointestinal radiography and endoscopy, the prognosis for advanced stages remains poor. For advanced disease, several chemosensitivity tests have been used for selection of an appropriate drug for adjuvant treatment after surgery. Some of which have been shown to confer a potential survival benefit in gastrointestinal cancer patients for whom an appropriate drug was selected by means of the test [6,9,10]. Retrospective analyses have shown survival in cancer patients treated with a 'tested' drug to be superior to that of patients treated with a standard drug [9,10]; however, there has been no prospective randomized controlled trial comparing survival between patients given a 'tested' drug, patients treated by surgery alone and patients treated by standard chemotherapy. It is still unclear whether patients treated with a 'tested' drug have improved survival after surgery. The second purpose of chemosensitivity testing is to avoid the use of any drug that may cause an adverse effect. In the case of gastric cancer, despite the fact that 'tested' drugs have only a 20–30% response rate when used as a single agent [11,12], we may expect that adjuvant use of an appropriate drug selected on the basis of chemosensitivity testing will prolong survival. Since the retrospective analysis suggested that treatment with an appropriate 'tested' drug had a potential survival benefit in advanced cases when compared to treatment with a standard drug, a prospective randomized controlled study comparing patients treated with a 'tested' drug and those treated with surgery alone will be critical for showing the survival benefit of chemosensitivity testing for adjuvant chemotherapy in patients with gastric cancer.

Chemosensitivity testing for gastrointestinal cancers developed because single agents showed less of a response rate than combination regimens and testing became necessary for selection of the most effective drugs in any one case. In current treatment practices for patients with gastric cancer, there is no standard regimen. It is still unclear whether the observed survival benefit

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obtained by chemosensitivity testing overrides the survival benefit that would be achieved by a standard regimen. In the process of incorporating new drugs including CPT-11 [13], S1, a new oral fluoropyrimidine [14], and taxanes [15,16] into new adjuvant chemotherapy regimens, the clinical relevance of chemosensitivity testing needs to be documented in terms of the potential and limitations of any survival benefit. Despite the fact that several chemosensitivity tests are used clinically with a potential method for individualized chemotherapy in cancer patients, the survival benefit of chemosensitivity testing is still unclear. We discuss the potential survival benefit and current limitations of chemosensitivity testing in patients with gastrointestinal cancer.

History of drug sensitivity testing

Although cancer chemotherapy has shown great progress since the introduction of nitrogen mustards for lymphoma in the 1940s, individual patients with histologically identical tumors do not always respond identically to the same drug regimen. In an attempt to individualize therapy, a number of *in vitro* assays have been developed to predict therapeutic response prior to chemotherapy. In the 1970s, the HTCA was applied to surgical specimens of solid tumors [1,17]. The HTCA is a growth assay in which fibroblast proliferation is inhibited by the use of a double-layer soft agar medium. Clonogenic assay is the traditional method for evaluating surviving cancer cells through clonogenic potential, but there are serious problems with clonogenic assays, including the disruption of normal cell-to-cell interactions and the possibility that true tumor stem cells may be largely non-dividing cells (G_0) [18]. Thus, clonogenic cells may largely represent cells that are not true stem cells. Because the success rate of primary culture with surgical specimens was low and many tumor cells are required for clonogenic assays, several modifications of culture techniques have been made including the monolayer MTT assay [2,3] and the HDRA, which use a combination of MTT colorimetry and tissue culture on a collagen gel sponge [6,9].

Cell viability in these assays is roughly classified according to the morphological changes and enzyme activities involved in cell survival. In the typical enzymatic estimation of viable cells, the succinic dehydrogenase inhibition test can be used; this was developed by Kondo in 1966 [19]. Subsequent modifications for evaluation of enzymatic activity in viable cancer cells yielded the MTT [2,3,20] and ATP [21,22] assays. The monolayer culture MTT assay is based on the reduction of MTT by mitochondrial succinic dehydrogenase, resulting in the production of the colored compound formazan, an enzymatic receptor; optical density is measured with a microplate reader. This assay can evaluate drug sensitivity more rapidly and simply, and yields a success rate of more than 90%. Because the cancer cells are assessed as a single suspension, however, cell–cell contact is eliminated [6]. The MTT assay is now applied mainly in clinical practice in Japan as an *in vitro* assay because of its convenience, rapidity and low cost in determining appropriate drugs for chemotherapy, but the test is significantly influenced by the presence of contaminating fibroblasts. Another disadvantage of monolayer culture is the decreased viability of control tumor cells during culture.

In contrast to *in vitro* assays, several *in vivo* assays reproducing the clinical situation, including the nude mouse isotope assay [23,24] and subrenal capsule assay [24–26], have been developed. Although improved sensitivity and specificity in surgical specimens provide potential clinical usefulness, the convenience and economics of such *in vivo* assays are not superior to those of *in vitro* assays. Because chemosensitivity tests need to be convenient, rapid and low in cost, *in vitro* assays may be more suitable for clinical practice. In considering *in vivo* chemosensitivity, a three-dimensional HDRA assay with soft agar may be more useful in choosing an appropriate drug. Several *in vitro* and *in vivo* chemosensitivity tests were developed and made available for clinical practice between 1970 and 1999, and the advantages and disadvantages of these tests have been described [27–29].

Several problems with chemosensitivity testing

Evaluation of chemosensitivity of tumor cells

Some chemosensitivity tests evaluate the sensitivity of cancer cells as well as non-cancer cells including macrophages, fibroblasts and stromal cells to various drugs. A major obstacle in evaluating sensitivity of cancer cells to drugs is that non-cancer cells are occasionally grown along with cancer cells in *in vitro* and *in vivo* assays, resulting in difficulty distinguishing the chemosensitivity of cancer cells. To avoid growth of non-cancer cells, serum-free medium is used for culture in the chemosensitivity tests. Some modified MTT assays that include removal of interstitial cells by Ficoll-Hypaque and Percoll discontinuous gradients [30] or culturing with serum-free media to inhibit the growth of non-cancer cells [31] produce good results in cases of gastrointestinal cancer. Although interaction of cancer cells with non-cancer cells in tumor growth mediated in a paracrine fashion cannot be estimated *in vitro*, and because interaction between cancer and non-cancer cells is important for the growth of solid tumors even under hypoxic and glucose starvation conditions, estimation of chemosensitivity for both including cancer cells and non-cancer cells is required. Indeed, induction of hypoxia and glucose starvation following treatment with an anticancer drug increases anti-apoptotic proteins such as Akt and NF-κB in non-cancer cells for paracrine tumor growth, resulting in more

resistance to anticancer drugs [32–34]. This hypothesis is supported by a previous report of the differential gene expression profile between cancer and non-cancer cells [35]. The differential sensitivity to anticancer drugs between cancer and non-cancer cells influences the susceptibility of the cell to apoptosis and the evolution of drug resistance of cancer cells in response to chemotherapy. In this regard, leukemia cells are more chemosensitive *ex vivo* than hematopoietic cells to cytotoxic drugs (this is the case with many drugs used against leukemia) and solid tumors are more resistant *ex vivo* than normal cells [36].

The other hindrance to chemosensitivity testing is reproduction of the clinical conditions in the assessment of drug sensitivity. The CD-DST was developed to eliminate the effects of fibroblasts in the cell culture on a collagen matrix, resembling *in vivo* cell growth cultured in a three-dimensional formation by embedding the cells in collagen gel [7,8]. Some advantages of this assay have been reported: the small number of cells required for each assay, a high primary culture success rate, the ability to maintain original growth morphologies of the cells, the inhibition of fibroblast proliferation, the exposure of tumor cells to physiological drug concentrations and the selective quantification of tumor cell colonies by image analysis [37]. Although the CD-DST is considered to be a reliable technique for determining tumor sensitivity to anticancer drugs [38], there are some clinical disadvantages: specimens must be taken into the culture system without contamination, it may take over 2 weeks for evaluation and the system is expensive.

The HDRA is an *in vitro* chemosensitivity test with a three-dimensional culture system that was first reported by Hoffman [4,5]. Conventional *in vitro* assays including MTT and ATP cannot be used to sufficiently evaluate tumor sensitivity to time-dependent drugs such as 5-fluorouracil (5-FU). Approximately 1 mm^3 of cancer tissue is cultured on a collagen gel without enzymatic tissue digestion, thereby cell–cell contact is maintained and long-term culture is possible under conditions resembling those *in vivo*. Modification of the original HDRA was carried out to assess cell viability by MTT assay instead of by autoradiography [6], and the modified HDRA has been used in the case of gastric and colorectal cancer, and other kinds of solid tumors. The clinical efficacy of chemotherapy has been shown to correlate strongly with HDRA data in solid tumors [9]. The modified HDRA is not superior to *in vitro* assays in terms of simplicity or cost.

Sensitivity and specificity

The greatest obstacle to clinical application of *in vitro* chemosensitivity testing by growth assay is the low rate of successful primary culture of human tumor cells, especially for gastrointestinal tract cancers, which contain abundant amounts of interstitial tissue. The HTCA method shows a 40–60% primary culture success rate for a variety of tumors [39,40], whereas the HDRA procedure improves the success rate to more than 80% for gastric cancer [9]. Similarly, CD-DST shows an 80% primary culture success rate for various tumors [41]. Since chemosensitivity testing contributes to the selection of an appropriate drug by predicting chemosensitivity of tumor tissues, assays should have high sensitivity as well as high specificity. The sensitivity and specificity is evaluated with clinical correlation between chemosensitivity testing and the response to the 'tested' drug, which means the true positive and true negative rate for the predictive accuracy of chemosensitivity testing. In general, drug sensitivity is evaluated by the IC_{50} value in the growth inhibition rate of drug-treated tumor cells compared to untreated cells. The use of more than 30–50% growth inhibition rate shows a high sensitivity in the evaluation of tested dugs; however, an arbitrarily assigned growth inhibition rate may not always reflect clinical response because clinical response needs to be based on log killed cells. In contrast, the specificity of chemosensitivity testing is high according to previous reports [31,38] because the response rate to anticancer drugs of gastrointestinal cancer cells is low due to constitutive drug resistance. These findings indicate that chemosensitivity testing is more suitable for identifying the wrong drug rather than the appropriate drug, even though there are only few drugs that produce a clinical response. Therefore, chemosensitivity testing may be useful not only for excluding the wrong drug or avoiding an adverse effect in the cancer patient, but also for avoiding wasted drug expenditure.

Correlation between drug sensitivity and clinical response

Previous reports have indicated a strong correlation between clinical drug sensitivity and the response to *in vitro* chemosensitivity testing in cases of gastrointestinal cancer and other solid tumors [10,42–45]. Correlation of HTCA results with clinical efficacy yields a true-positive rate, which means that the drug sensitivity is observed both *in vitro* and clinically in 60% of cases, and a true-negative rate of 85%, which means that non-sensitivity is observed both *in vitro* and clinically in 85% of cases [38]. For gastric cancer patients with tumors sensitive to chemotherapy, as assessed by HDRA, the overall and disease-free survival rates were significantly higher than for those with tumors resistant to drugs and the assay-determined tumor chemosensitivity is shown to be a prognostic factor [6,9]. Similarly, the survival rate of patients treated with a 'tested' drug is higher than that of patients treated with an inappropriate drug or surgery alone for gastrointestinal tract cancer or other solid tumors [46–53] (Table 1). These findings suggest that

Table 1 Reported survival benefit in gastrointestinal cancer patients treated with a 'tested'[a] drug in comparison to a standard drug during postoperative adjuvant chemotherapy

Author and year	Chemosensitivity test	Type of tumor	No. of patients evaluated	Treatment regimen	Survival benefit (*p*-value)	Evidence level[b]
Kubota *et al.*, 1995 [9]	HDRA	gastric cancer (stage III, IV)	128	MMC + UFT	sensitive versus resistant (OS, $p=0.001$, log-rank test; $p=0.0007$, Wilcoxon test)	IIb
Furukawa *et al.*, 1995 [6]	HDRA	gastric cancer (stage III, IV)	32	MMC + UFT	sensitive versus resistant (OS, $p<0.005$, log-rank test)	III
		colorectal cancer (stage III, IV)	29	5-FU	sensitive versus resistant (RFS, $p<0.05$, log-rank test)	
Abe *et al.*, 1999 [47]	MTT	gastric cancer (stage III, non-scirrhous)	28	MMC or ADM, CDDP, UFT	sensitive versus resistant (OS, $p<0.05$)	III
Kabeshima *et al.*, 2002 [48]	MTT	colorectal cancer (Dukes C, D)	200	MMC, CDDP, UFT	sensitive versus resistant or versus surgery alone (OS, $p=0.0158$, $p=0.0004$, log-rank test)	III
Yamaue *et al.*, 2002 [51]	MTT	pancreas cancer (stage I–IV)	14	MMC, CDDP, 5-FU, ADM IAI	sensitive versus resistant (OS, $p<0.05$, Wilcoxon test)	III
Kubota *et al.*, 2003 [49]	MTT	gastric cancer (stage III, IV)	50	ND	sensitive versus resistant or versus surgery alone (OS, $p<0.01$)	III

[a]'Tested' drug is a drug identified by chemsensitivity testing of surgical specimens.
[b]Evidence level is determined according to AHCPR (Agency for Health Care Policy and Research, USA) criteria. MMC, mitomycin C; ADM, adriamycin; CDDP, cisplatin; IAI, intra-arterial infusion; ND, not described; OS, overall survival; RFS, recurrence-free survival.

since the overall accuracy has improved by modifications to the conventional chemosensitivity tests, such testing may prolong the survival of patients treated adjuvantly with an appropriate drug. The implication of a survival benefit by chemosensitivity testing is based on retrospective analysis and, in part, on a prospective, non-randomized pilot study. It is still not clear whether survival of patients treated with a 'tested' drug is improved over survival of patients treated with a standard chemotherapy drug or whether survival of patients treated with drug-resistant tumor can be improved. It is possible that patients responsive to chemotherapy are affected by standard chemotherapy toward increased survival, whereas patients who are not responsive to chemotherapy are not affected by a chemotherapy regimen selected on the basis of by chemosensitivity testing (Fig. 1). Although selection of an appropriate drug by chemosensitivity testing can affect the drug-sensitive tumor, the survival benefit in selected patients may be equal to that of patients who respond to standard chemotherapy.

Tumor heterogeneity

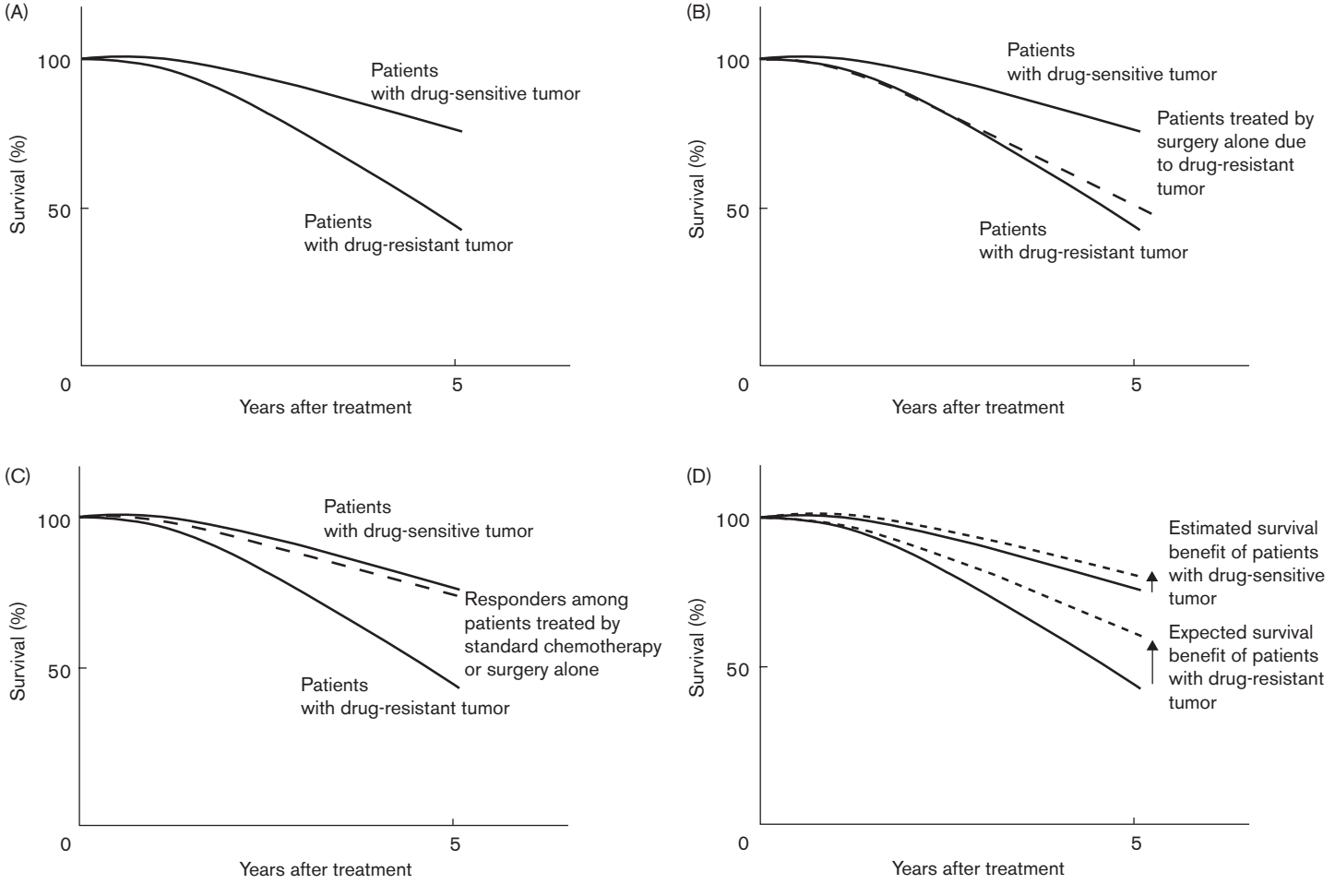
Systemic treatment of breast and gastrointestinal tumors has been based on physicians' empirical judgment and relies on data obtained from clinical trials. Because the findings of clinical trials represent average therapeutic responses of patient groups, individual differences are not accounted for [54]. The effectiveness of current therapeutic approaches is probably limited by tumor heterogeneity, at least for certain types of tumor [55,56]. A previous report indicated that sensitivity profiles of two different areas of the same primary tumor were quite similar, whereas when one sampled a primary tumor and its metastasis for drug sensitivity test, the agreement was poor. In addition, agreement was also poor in drug-sensitivity profiles of one metastasis versus another [55]. These observations reflect some of the false-positives and -negatives noted in clinical correlation trials, and indicate that adjuvant chemotherapy where chemosensitivity of the primary tumor can be determined may not predict chemosensitivity of the micrometastasis. Nevertheless, the demonstration of considerable heterogeneity of chemosensitivity between the tumors of esophageal and gastric carcinoma may suggest individualization of chemotherapy by chemosensitivity testing for improvement of patient response and survival [57].

Future perspectives

Scientific validation

Previous retrospective analyses have shown that survival of patients treated with the 'tested' drug is better than that of patients treated with the standard drug. Further, some preliminary prospective studies without randomization suggested that the use of chemosensitivity testing might confer a survival benefit by excluding drugs that would cause an adverse effect in cancer patients. Indeed, avoiding use of the wrong drug is important in terms of both cost and adverse effects. When resistance is suspected, the patient is usually treated with surgery

Fig. 1



(A) A model of the estimated survival benefit for drug-sensitive and -resistant tumors in adjuvant chemotherapy for gastrointestinal cancer. (B and C) Survival of patients with a good prognosis due to a drug-sensitive tumor will be affected by the appropriate drug to some extent, whereas survival of patients with a poor prognosis due to a drug-resistant tumor cannot be improved by standard chemotherapy regimens. The survival benefit for patients treated with a 'tested' drug may differ significantly from that of patients treated by standard chemotherapy or surgery alone. (D) Targeting prognostic factors in patients with drug resistance will be critical to increasing overall survival in the total population of patients treated with adjuvant chemotherapy.

alone. Comparisons of gastrointestinal cancer patients treated with a 'tested' drug and those treated with surgery alone suggest that survival is improved in patients treated with the drug. The design of chemotherapy regimens with appropriate drugs needs scientific validation. Despite the reported advantages and disadvantages associated with each chemosensitivity test, tests such as the MTT, HDRA and CD-DST have been optimized for clinical use in terms of convenience, rapidness and economics. Additional sufficient and scientific validation studies will need to proceed to the subsequent randomized controlled study.

Survival benefit potential and limitations with chemosensitivity testing

In considering adjuvant chemotherapy after surgery for gastrointestinal cancer, despite the fact that survival of patients with drug-responsive disease can be improved by standard chemotherapy, this population may be the same as that of patients with drug-sensitive tumors. The potential survival benefit for patients with drug-sensitive tumors will be marginal compared to the total population treated with adjuvant chemotherapy. In fact, identification of drug-resistant tumors by chemosensitivity testing is useful for excluding ineffective drugs and avoiding the adverse effects of chemotherapy. The limited survival benefit may be explained by the model shown in Figure 1. In general, survival benefit must be evaluated on the basis of prognosis: good or poor. The estimated survival benefit in patients with a good prognosis, i.e. considered to be drug responsive, may be marginal, whereas the survival benefit in patients with a poor prognosis cannot be improved by conventional combination chemotherapy. Thus, the survival benefit conferred by a 'tested' drug in responders with a good prognosis may be limited, but whether the survival benefit is increased in non-responders with a poor prognosis is still uncertain. Improvement in the survival of patients with a poor prognosis is necessary to increase overall survival in the total population treated with adjuvant chemotherapy and particular molecular targeting therapies need to be developed to overcome drug resistance in patients with a poor prognosis.

Previous reports have indicated to that a predictive factor is different from a prognostic factor in cancer patients [58]. Survival related to prognostic factors cannot be modulated by anticancer drugs, whereas survival related to predictive factors can be improved by the specific anticancer drug associated with each predictive factor. For example, although overexpression of HER-2 is a factor conferring a poor prognosis in patients with breast cancer, HER-2 is also an important factor predictive of the response to anti-HER-2 antibody, herceptin, or combination therapy with taxanes [59]. Further, recent reports also indicate that overexpression of HER-2 might be a predictive factor for responsiveness to treatment with anthracyclines and tamoxifen [60,61]. In gastrointestinal cancer, despite the fact that thymidylate synthase (TS) and thymidine phosphorylase (TP) are important prognostic factors associated with the responsiveness of 5-FU and 5′-DFUR [62,63], the survival benefit of adjuvant chemotherapy targeted to these factors has not been yet documented. Thus, further identification of new predictive factors associated with prognosis factor in cancer chemotherapy will be required for improved survival of patients with solid tumors, including gastrointestinal cancer. Given that new anticancer drugs including CPT-11, S1, taxanes and gemcitabine have been developed for clinical use, new combination therapies such as S1 + CPT-11, S1 + docetaxel and other combinations will likely increase therapeutic efficacy in cases of gastrointestinal cancer. An improved survival benefit from adjuvant chemotherapy can be expected only by development of new combination regimens and new molecular targeted agents in patients with gastrointestinal cancer.

Individualized chemotherapy

For individual patients, the most promising drug could be chosen and an optimal treatment strategy developed. The conventional cellular assay is broadly applicable for substance pre-selection in drug discovery and tumor therapy, and several pre-selection approaches have been evaluated for their applicability. Despite the development and clinical application of *in vitro* tests held over the years, chemosensitivity tests have failed to achieve broad acceptance for routine clinical use. Molecular assays are a recent development, and the feasibility of their use in clinical testing and treatment cannot be predicted as yet. Techniques for producing gene expression profiles for individual patients may personalize chemotherapy. A previous report of a genomics-based approach to the prediction of drug response described an algorithm for classification of cell line drug sensitivities based on gene expression profiles alone. Gene expression-based classifiers of sensitivity or resistance for 232 compounds were generated and evaluated on independent sets of data [64]. Prediction of chemosensitivity must be extended beyond cell line models to include primary patient materials and, to date, few clinical studies have reported clinically relevant gene expression patterns extracted from tumor samples [65–69]. Although the previous studies indicate the potential for screening samples for genetic determinants of chemosensitivity, the current individual gene expression profiles do not support individualized chemotherapy protocols. Another approach to determining predictive factors for drug sensitivity is made clear through the example of sensitivity to 5-FU, which can be assessed by *in vitro* or *in vivo* assay at the cellular level, because several factors affecting chemosensitivity have been identified at the molecular level.

Factors contributing to 5-FU sensitivity, such as TS, thymidine phosphorylase (TdR-Pase) and dihydropyrimidine dehydrogenase (DPD), have been found [70–73], and the clinical significance of these factors for prediction of 5-FU sensitivity has been documented. In these reports, the low mRNA expression level of three genes, TS, TP and DPD, which is measured by quantitative RT-PCR in pretreatment tumor biopsies and post-treatment surgical specimens, can predict the responsiveness of 5-FU-based therapy in colorectal cancer. The combined assessment of these factors increases the predictive accuracy for responsiveness of 5-FU-based therapy. Standardization of the technique for the assessment and the best cut-off points of these predictive factors are required for clinical application. Thus, identification of critical factors contributing to drug sensitivity may be more useful for the prediction of sensitivity with tumor biopsy samples or surgical specimens. The clinical relevance of these factors for individualized chemotherapy in comparison to standard chemotherapy should be evaluated by randomized controlled studies. These critical factors contributing to drug sensitivity, which are determined by retrospective correlation analysis with clinical samples, are sometimes different from DNA microarray analysis. Indeed, it has been reported that specific gene expression levels following treatment with anticancer drugs including genes related to cell proliferation, angiogenesis and apoptosis differ between responders and non-responders. However, the most likely clinically important genes are not included in the gene expression profiles compared between responders and non-responders. Thus, although the initial DNA microarray analysis and other molecular methods do not override the conventional cellular assay method for preselection of the appropriate drug for cancer chemotherapy, distinguishing individual responsiveness at the molecular level as well as the cellular level will be critical for individualization of chemotherapy in patients with gastrointestinal cancer. Recent advances of cDNA microarray analysis suggest that the differential expression profiles between responders and non-responders in chemotherapy for esophageal and other cancers have been identified by discovery and validation studies [74–76]. In addition, the clinical utility of cDNA microarrays for prediction of responsiveness in chemotherapy is under investigation in prospective randomized controlled studies. If these observations are confirmed, this could represent a successful clinical application of cDNA microarrays to a challenge for individualization that could not be addressed adequately with previous technologies. It is worth noting that the standardization of cDNA microarrays is very important for routine clinical application, because current microarray platforms exist that use distinct sets of genes and employ different hybridization and signal detection methods [77]. Further proof of principle studies in molecular analysis for individualization of chemotherapy are needed.

Clinical trials

Several retrospective correlation analyses and some prospective validation-like studies have shown a potential survival benefit for drug sensitivity testing in gastrointestinal cancers and other solid tumors. After a scientific validation study, a randomized, controlled phase III study comparing individualized chemotherapy against standard chemotherapy is needed to establish a true survival benefit. New chemotherapy regimens that include a molecular targeting agent or that target specific prognostic factors associated with predictive factors may also improve the survival of patients with gastrointestinal cancer. Because the survival benefit determined for standard chemotherapy is an average group value, it does not really reflect individual clinical responses to chemotherapy. Thus, the potential survival benefit and its limitations in relation to chemosensitivity testing and molecular analyses may be explained by the current efficacy of anticancer drugs in the treatment of gastrointestinal cancers. The design of a clinical trial of individualized chemotherapy must include standardization and simplicity of the chemosensitivity testing and molecular analysis methods as well as global and multicenter trials.

Conclusion

Oncologists have tried to carry out chemosensitivity testing *in vitro* and *in vivo* on animal models. Despite the fact that correlation analyses have shown a potential survival benefit in patients with drug-sensitive tumors, chemosensitivity testing is not widely accepted. The major reason for this lack of acceptance appears to be lack of sufficient data obtained in the clinical setting. Scientific validation studies and the standardization of chemosensitivity testing can proceed to randomized controlled phase III studies for statistical confirmation of a clinical benefit. The establishment of assay methodologies will be important if we are to break through the appearance of a limited survival benefit in patients with gastrointestinal cancer. Ultimately, chemotherapy based on individual cellular and genetic differences may be on the horizon. The potential survival benefit of individualized chemotherapy based on chemosensitivity testing and other molecular analysis to distinguish drug response needs to be confirmed by randomized controlled study in comparison with standard chemotherapy. Overriding the marginal effect of adjuvant chemotherapy with new molecular targeting agents and other agents influencing drug response is needed.

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