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Pharmacokinetics and pharmacogenomics in gastric cancer chemotherapy ☆

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ABSTRACT

Despite extensive efforts, treatment of gastric cancer by chemotherapy, the globally accepted standard, is yet undetermined, and uncertainty remains regarding the optimal regimen. Recent introduction of active "new generation agents" offers hope for improving patient outcomes. Current chemotherapeutic trials provided several regimens that may become a possible standard treatment, including docetaxel/cisplatin/5-FU (TCF) and cisplatin/S-1 for advanced and metastatic cancer and S-1 monotherapy in the adjuvant setting. Along with the development of novel active regimens, individual optimization of cancer chemotherapy has been attempted in order to reduce toxicity and enhance tumor response. Unlike the rare and limited contribution of pharmacokinetic studies, pharmacogenomic studies are increasing the potential to realize the therapeutics against gastric cancer. Despite the limited data, pharmacogenomics in gastric cancer have provided a number of putative biomarkers for the prediction of tumor response to chemotherapies and of toxicity.

Contents

1. Introduction

Gastric cancer is the fourth most common cancer and the second most frequent cause of cancer-related mortality worldwide, accounting for approximately 930,000 new cases and 700,000 deaths annually [1,2]. In gastric cancer, radical surgery remains the standard form of therapy that has curative intent, although chemotherapy has been intensively studied in a variety of settings as a most potent treatment option to improve the poor survival rate [3–5]. A series of trials has produced evidence that chemotherapy increases survival, but a globally accepted standard chemotherapy is yet undetermined, and uncertainty remains regarding the choice of the optimal regimen.

Recently, several active agents have been introduced to gastric cancer therapy: the taxanes, irinotecan (CPT-11), oxaliplatin, S-1, capecitabine, and, more recently, biological agents such as cetuximab and bevacizumab [3–7]. The advent of new regimens of these new generation agents offer hope for improving patient outcomes, and rapid advances in genomics present the opportunity to establish a novel chemotherapeutic strategy, personalized medicine, which would allow the selection of optimal therapy and dose for each

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Table 1
Current chemotherapeutic option and supporting Phase III study in gastric cancer.

Treatment	Phase III study (Pts.No)	Main results
Neoadjuvant setting		
ECF [11]	MAGIC trial (503)	⊕ Higher PFS and higher likelihood of OS in ECF group (vs. surgery alone)
Adjuvant setting		
45 Gy radiation + 5-FU/FA [10]	INT-0116/SWOG 9008 study3 (556)	⊕ Higher RFS and OS in stage IB to stage IV (M0) patients underwent complete rsection (vs. surgery alone)
S-1 monotherapy [12]	ACTS-GC (1059)	⊕ Higher RFS and OS in stage II/III patients who underwent complete resection with D2 dissection vs. surgery alone) ⊕ Less Grade 3/4 toxicity than other regimens.
Metastatic stage		
TCF [17,18]	V-325 study	⊕ Higher RR, TTP and OS (vs. CF)
S-1 monotherapy [20]	JCOG 9912	⊕ Higher likelihood of OS to continuous 5-FU ⊕ Compatible OS with CPT-11/cisplatin and significant less Grade 3/4 toxicity
S-1/cisplatin [21]	SPIRITS trial	⊕ Higher PFS and OS (vs. S-1 monotherapy)

ECF, epirubicin/cisplatin/5-FU combination; TCF, doceraxel/cisplatin/5-FU combination; OS, overall survival; RFS, relapse-free survival; RR, response rate; TTP, time to tumor progression; PFS, progression-free survival.

individual based on molecular profiles of both the tumor and the patient [8,9].

2. Current chemotherapeutic strategies for gastric cancer

Although personalized medicine is an attractive strategy with great potential, at present, establishment of the first golden standard regimen takes priority over the development of novel therapeutics against gastric cancer. A great deal of effort has been directed towards development of the best regimen in various treatment settings (Table 1) [3–7].

2.1. Adjuvant chemotherapy

In the US, chemoradiation therapy (CRT) has been evaluated as the focus for an adjuvant treatment. The Phase III Intergroup trial (INT-0116/SWOG 9008 study3) is noteworthy among the conducted trials [10]. This study demonstrated that adjuvant 45 Gy radiation therapy with combined chemotherapy of 5-fluorouracil (5-FU) and folinic acid (FA) improved relapse-free survival (RFS) and overall survival (OS) in 556 patients with completely resected stage IB to stage IV (M0) adenocarcinoma of the stomach and gastroesophageal junction. Based on the results, CRT after potentially curative surgery for node-positive patients is now regarded as a standard treatment in the US.

Perioperative (pre- and post-operative) chemotherapy without radiation therapy has also been intensively studied. In UK, a large phase III study, The Medical Research Council Adjuvant Gastric Infusional Chemotherapy (MAGIC) trial, was conducted to evaluate the efficacy of ECF combination regimen (epirubicin, cisplatin, and continuous infusion of 5-FU) as a perioperative adjuvant therapy (before and after surgery) [11]. The study, in which 503 patients with stage II or higher adenocarcinoma of the stomach or of the lower third of the esophagus were enrolled, demonstrated a significantly higher progression-free survival (hazard ratio [HR] for progression, 0.66; 95% confidence interval [CI], 0.53–0.81; $P<0.001$) and OS (HR for death, 0.75; 95% CI, 0.60–0.93; $P=0.009$) in the perioperative chemotherapy group compared with the group of patients who have undergone surgery alone. The study also validated the feasibility of the regimen in a preoperative adjuvant setting, which led to the current European recognition of neoadjuvant chemotherapy as a standard treatment option in gastric cancer. Neoadjuvant CRT remains under clinical evaluation.

The recent Phase III studies also supported the role of post-adjuvant chemotherapy in gastric cancer. The ACTS-GC conducted in Japan, in which a total of 1,059 patients were enrolled, evaluated the adjuvant efficacy of S-1 monotherapy compared with surgery alone, with OS as the primary end point, and showed the benefit of S-1 monotherapy as postoperative adjuvant chemotherapy for stage II/III patients who underwent D2 dissection [12]. Three-year OS after S-1 monotherapy was 81.1%, while that of the surgery alone group was 70.1% ($P=0.0015$), and Grade 3/4 adverse events were less frequent than other regimens. RFSs after a 3-year follow-up of S-1 and of the surgery alone group were 72.2% and 60.1%, respectively ($P=0.0001$). The feasible and effective adjuvant therapy with S-1 monotherapy after curative surgery is regarded as the standard of care of stage II/III gastric cancer patients in Japan. The Italian Gruppo Oncologico dell'Italia Meridionale (GOIM) study also found positive results for epirubicin/etoposide/5-FU/FA) in patients who underwent D1, D2 dissection [13].

2.2. Chemotherapy in advanced, metastatic, and recurrent gastric cancer

For advanced, metastatic, and recurrent gastric cancer, 5-FU- and/or cisplatin-based combinations remain the mainstay of treatment [3–7]. A meta-analysis by Wagner et al. showed that conventional cytotoxic chemotherapy ($n=103$) can improve OS (HR = 0.39, 95%CI: 0.28–0.52, $P<0.00001$), quality of life (HR = 2.07, 95% CI: 1.31–3.28, $P=0.002$) and symptom-free period (HR = 2.33, 95% CI: 1.41–3.87, $P=0.001$) compared with Best Supportive Care (BSC) ($n=81$). The analysis also demonstrated a survival benefit of cytotoxic combinations ($n=836$) over single-agent ($n=636$) chemotherapy (HR = 0.83, 95% CI: 0.74–0.93, $P=0.001$) and an advantage of cisplatin based on OS [14]. Indeed, both CF (cisplatin/5-FU) and ECF (epirubicin/cisplatin/5-FU) are regarded as the most potent treatment option in various countries, especially in the US and Europe.

Nevertheless, with these "classical" combinations, median survival time (MST) does not exceed 7-10 months. Current studies have focused on "new generation agents," such as the taxanes (paclitaxel and docetaxel), irinotecan, oxaliplatin, new oral fluoropyrimidines, and biological agents [3–7]. Several combinations of these agents, with response rates of up to 65% reported in phase II studies, have been investigated in randomized phase II/III studies [15,16].

To date, docetaxel combinations, especially a docetaxel/cisplatin/5-FU (TCF) regimen, are likely crucial in various regimens. In the phase III V-325 study, the triple agent therapy was superior to CF in terms of response rate (37% vs. 25%; 95% CI: 30.3-43.4 vs. 19.9-31.7, chi-square $P=0.0106$), time to progression (TTP; 5.6 months vs. 3.7 months; HR = 1.47, 95% CI: 1.19–1.82, risk reduction 32%, log-rank $P=0.0004$), and survival (risk reduction 23%, HR = 1.29, 95% CI: 1.0–1.6, log-rank $P=0.0201$) in patients with metastatic gastric cancer [17,18]. TCF triplet is recognized as a new reference regimen for advanced gastric cancer in the US and Europe. However, the triplet regimen appeared to be highly toxic compared with other regimens. The optimizing studies of the original regimen, in terms of both the efficacy and safety, are currently in progress [19].

New oral 5-FU analogs and prodrugs such as S-1 and capecitabine are also attractive alternative agents for combination with other anticancer agents. Recent phase III trials have focused on the use of oral 5-FU, especially for S-1, and demonstrated these pivotal activities. A three arm Phase III study [Japan Clinical Oncology Group (JCOG) 9912]

Table 2
Putative biomarker of chemotherapeutic response in gastric cancer.

Drug or therapy	Gene	Genomic marker (expression and polymorphism)	Indicated phenotype
Predictive marker of therapeutic efficacy			
Fluoropyrimidines or fluoropyrimidine-based therapy	*TYMS*	High expression in tumor, 6 bp insertion in 3′UTR, 3R VNTR in 5′UTR, G>C in 3R VNTR allele in 5′UTR (3RG)	Poor response to the therapy
	DPYD	High expression in tumor	Poor response to the therapy
	MTHFR	677C>T	Increased drug sensitivity
	OPRT	Low expression in tumor	Poor response to the therapy
Platinum or platinum-based therapy	*ERCC1*	High expression in tumor, 118C>T	Poor response to the therapy
	XRCC1	194C>T, 399G>A	Increased response to the therapy
	XPD (ERCC2)	312G>A	Increased response to the therapy
	XRCC3	241T>C	Increased response to the therapy
	GSTP1	Low expression in tumor, 105 A>G	Increased response to the therapy
	GSTM1	Deletion (null allele)	Increased response to the therapy
	GSTT1	Deletion (null allele)	Increased response to the therapy
Irinotecan, TXL, platinums	*ABCB1*	High expression in tumor, 1236C>T, 2677G>T/A, 3435C>T	Resistant to the drug
PLF-based therapy	*TYMS*	*TYMS* (2R VNTR: 2R/2R)	Longer OS of the patient
5-FU/LV-based therapy	*TYMS*	*TYMS*(G>C IN 3R VNTR and +6 bp allele)	Shorter OS and DFS of the patient
5-FU/LV/cisplatin and CF	*TYMS, GSTP1*	*TYMS* (2R/2R, 2R/3RC, 3RC/3RC) + *GSTP1*105 A>G	Longer OS and PFS of the patient
Predictive marker of toxicity			
Fluoropyrimidines	*DPYD*	IVS14 + 1G>A (*DPYD**2A)	Severe, life-threatening toxicity
Irinotecan	*UGT1A1*	-41_-40dupTA (TA7/TA7) (*UGT1A1**28)	Increased toxicity

UTR, untranslated region; bp, base pair; VNTR, variable number of 28-bp tandem repeats; TXL, paclitaxel; PLF, cisplatin/5-FU/LV; OS, overall survival; RFS, relapse-free survival; RR, response rate; TTP, time to tumor progression; PFS, progression-free survival.

comparing continuous infusion 5-FU, S-1 monotherapy, and CPT-11/cisplatin with the primary end point of OS showed that S-1 monotherapy seemed superior to continuous 5-FU and almost comparable with CPT-11/cisplatin combination, with significantly less incidence of grade 3, 4 toxicity than CPT-11/cisplatin [20]. The SPIRITS trial comparing S-1 monotherapy with S-1/cisplatin combination demonstrated that S-1/cisplatin combination significantly improved OS (11 vs. 13 months; HR 0.774; 95% CI 0.610-0.980; P = 0.0366) and PFS (4 vs. 6 months; HR 0.57; 95% CI 0.437-0.734; P < 0.0001) at a median follow-up of 34.6 months [21]. The phase III First-Line Advanced Gastric Cancer (FLAGS) trial, designed to compare CF with S-1/cisplatin, is currently in progress in North and South America, Australia, and Europe [22].

The incorporation of biological agents, such as bevacizumab and cetuximab, into combination regimens is another innovative approach, and the best partner of these agents is now under intense investigation [3–7].

3. Optimization of the therapy via pharmacokinetic evaluation

It was reported that 21% of the evaluable drug products between 1980 and 1999 underwent dosage changes after marketing approval [23]. Optimization of therapy with relatively narrow efficacy profiles and adjustment for high interpatient variability on a routine basis during the therapy are of utmost importance in cancer chemotherapy [24]. The information on pharmacokinetic properties is essential for adjusting treatment doses and schedules for individuals even when the initial treatment is unsatisfactory due to excessive toxicities or other complications.

Despite the suggested clinical benefit, the application of pharmacokinetics to the optimization of chemotherapy is restricted to narrow limits, and therapeutic drug monitoring is not routinely used in practical chemotherapy. The phramacodynamic profiles, in terms of both toxicity and efficacy, are generally used as a more practical guide for the optimization. As stated earlier, several attempts to modify the original DCF regimen to be a possible standard treatment are under way in order to improve its remarkable toxicity profile [19]. Even so, pharmacokinetic analysis and data obtained in the early development stages of the regimen are unlikely to have great impact on the optimization. To our knowledge, there are currently few reports demonstrating the pivotal role of pharmacokinetic evaluation in optimization of chemotherapy in gastric cancer.

Combination regimens are a mainstay in gastric cancer chemotherapy as well as in other cancers, which makes it difficult to determine the therapeutic ranges of individual drugs in a combination. The concentration-response relationship for a single drug is not always the same as when that drug is used in a combination.

However, recent reviews have suggested the transferability of pharmacokinetics to the bedside: Mielke described a possible individualized pharmacology with paclitaxel, one of the key drugs in gastric cancer chemotherapy, and showed hat prolonged exposure to paclitaxel concentrations exceeding the thresholds of 0.05 or 0.1 μmol/l was predictive for neutropenia [25]. Hurria and Lichtman provided an overview of pharmacological studies on anticancer therapies in older patients and showed an age-related decrease in clearance for several anticancer agents such as paclitaxel, etoposide, etoposide and cisplatin, and doxorubicin, indicating the important role of pharmacokinetic analysis in determination of the optimum treatment for the growing population of older cancer patients [26]. Application of pharmacokinetic analysis to chemotherapy optimization may be of substantial clinical benefit, but no definitive way to exploit the full power of the suggested benefit has yet been established, at least in gastric cancer chemotherapy.

4. Optimization of the therapy via pharmacogenomic evaluation

Differing from the pharmacokinetic approach, pharmacogenomics is increasingly recognized as the most powerful approach to optimize the therapy and the treatment dose for individuals [27–30]. Increasing amounts of evidence have promoted clinical application of pharmacogenomics. The FDA has validated these possible biomarkers and provided the information in the corresponding FDA-approved drug labels, describing three recommendation levels for testing: "required," "recommended," and "information only" (http://www.fda.gov/cder/genomics/genomic_biomarkers_table.htm) [31]. Despite still being highly challenging, the day of practical pharmacogenomics at the bedside has arrived, offering new potential for gastric cancer chemotherapy, among other illnesses. Table 2 lists the putative biomarkers whose clinical significance in gastric cancer and/or other malignancies have been demonstrated in more than two reports after a search through the literature on gene or polymorphism-drug sensitivity (or resistance) and toxicity of each drug on the National Library of Medicine's PubMed.

4.1. Pharmacogenomics of chemotherapeutic efficacy

At present, there is no FDA-approved predictive biomarker of efficacy for drugs commonly used in gastric cancer chemotherapy,

except C-KIT expression for imatinib mesylate in gastrointestinal stromal tumors [31]. Even so, advances in pharmacogenomics against gastric cancer have provided a number of putative candidate markers for the prediction of tumor response to chemotherapies [32–34].

Thymidilate synthase (TS) and dihydropyrimidine dehydrogenase (DPD) have been the foci of predictive biomarkers for 5-FU efficacy [27–32]. TS, the rate-limiting enzyme in de novo pyrimidine biosynthesis, is a target enzyme of 5-FU, and DPD is the initial rate-limiting enzyme in the degradation of 5-FU, with over 80% of an administered dose of the pyrimidine analogue being inactivated via this enzyme-mediated catabolic pathway. Recent evidence indicates that elevated TS and/or DPD in tumors, in both mRNA and protein levels, are associated with clinical resistance to 5-FU and consequently with poor outcome of the patients receiving 5-FU therapy [32–37]. These findings led us to focus on the regulatory mechanisms of these genes. Although the regulatory mechanisms of *DYPD* expression remain controversial [38,39], high *TYMS* expression is now well known to be associated with several polymorphisms, including polymorphism of 6-base pair (bp) insertion (6+/6+ genotype) in the 3′ untranslated region (UTR), triple 28-bp tandem repeat (VNTR; >3, 3R instead of 2, 2R) in the 5′ promoter enhancer region (TSER) and a G>C polymorphism [3G-containing genotypes (2R/3G, 3C/3G, 3G/3G)] in the 3R VNTR allele [32–37,40–43]. These genotype-phenotype correlations, and, furthermore, the association of several combinations of these polymorphisms such as *TYMS* high (3RG and +6 bp) expressing alleles with the 5-FU resistant phenotype, have also been shown in gastric cancer [44,45]. Though the specific action remains unknown recent findings have also indicated the possible correlation of 5-FU resistance with low gene expression of orotate phosphoribosyl transferase (OPRT), which is involved in the active conversion of 5-FU to FUMP in the presence of 5-phosphoribosyl-1-pyrophosphate [46,47]. Expression of *DPYD* and *OPRT* is likely a potent marker in clinical tumor responses to 5-FU; the expression level and/or the polymorphisms regulating *TYMS* expression are the most prominent candidates of biomarkers for 5-FU tumor response at present.

Several nucleotide excision repair enzymes and phase II detoxification enzymes such as the glutathione-S-transferases (GSTs) appear to be a putative determinant for platinum resistance [27–30]. Among a number of genes involved in the altered DNA repair mechanisms, the excision repair cross complementing 1 (*ERCC1*) gene, the X-ray cross complementing group 1 (*XRCC1*) gene, the xeroderma pigmentosum Group D (*XPD* or *ERCC2*) gene, and the X-ray cross complementing group 3 (*XRCC3*) gene are known to play important roles in DNA repair [48–51]. Indeed, expression of these genes and their product proteins has been shown to be associated with poor clinical outcome to platinum-based chemotherapy, including gastrointestinal malignancies [27–30]. A number of putative functional polymorphisms in these genes are under investigation for their predictive role in patients [52]. Several genetic variations of GST families, such as a SNP substitution of A>C at codon 105 in *GSTP1* and homozygous deletions in *GSTT1* or *GSTM1*, are suggested to also relate to the polymorphisms of the enzyme function: the former diminishes GSTP1 enzyme activity, and the latter leads to an absence of enzymatic activity of GSTT1 or GSTM1 [53–55]. The predictive roles of these polymorphisms in platinum response are now intensively studied.

Tumor response to taxanes is possibly regulated by the metabolizing enzymes-*CYP2C8*, *CYP3A4* and *CYP3A5*-, and a cellular transport, *ABCB1* [27–30,56,57]. No definitive biomarkers for the tumor response, however, have been identified to date. A predictive biomarker of irinotecan (CPT-11) response is also still unknown [27–30,58,59]. A variety of factors involved in CPT-11 pathway has been clarified, including the drug target topoismerase I gene, the carboxyesterase genes (*CES*s) as activation enzymes of the prodrug, the metabolism enzyme genes *CYP3A4* and *3A5*, uridine diphosphate glucuronosyltransferase 1A1 (*UGT1A1*), which glucuronidates the active form SN-38 to its inactive metabolite SN-38G, and efflux transporters *ABCB1* and *ABCB2*. All of them have predictive potential of CPT-11 response, but none of these factors alone is consistently critical in the drug response. The most significant progress made in CPT-11 pharmacogenomics is in predicting toxicity.

Recent pharmacogenomic studies investigated a set of putative biomarkers for each drug used in the combination, with the hypothesis being that key sensitivity markers for each component drug could allow us to predict therapeutic response to the combination therapy. Current evidence provided by these multi-gene pharmacogenomics indicates that *TYMS* polymorphisms and *GSTP1* variation play prominent roles in responses, respectively, to the 5-FU based regimen and platinum-containing therapy in gastric cancer. Ott et al. investigated the cisplatin/leucovorin/5-FU (PLF) regimen in the neoadjuvant setting and demonstrated that increased survival in a total of 135 patients was significantly associated with the 2R allele in *TYMS* promoter region [60]. Ruzzo et al. investigated 13 polymorphisms within 9 genes in 175 patients with advanced gastric cancer receiving 5-FU/cisplatin chemotherapy and demonstrated a significant association of chemoresistance and poor survival with *TYMS* 5′-UTR 3G-genotype, leading to low *TYMS* expression and/or *GSTP1* 105 A/A homozygous genotype, with the shortest median PFS and OS in the patients with both risk genotypes and best clinical outcome in the patients with "low producers of *TYMS*" [61]. Kawakami et al. investigated a set of polymorphisms in the *TYMS* in 90 patients receiving 5-FU/folinic acid-based regimens in the adjuvant setting and demonstrated that the patients with a combination of high *TYMS* expressing alleles had shorter overall survival (OS) and disease-free survival (DFS) [44]. Lu et al. investigated FU/calcium folinate associated with oxaliplatin or hydroxycamptothecin or cisplain or paclitaxel in 106 metastatic gastric cancer patients and demonstrated higher response rate in patients with at least one *TYMS*-6 bp allele [62].

Multiple genes are involved in the mechanisms with complex interplay. Despite still being in the investigational phase, attempts to predict tumor response using expression profiles of multiple key genes have been also intensively performed in various malignancies, including gastric cancer [27–30,45,63,64]. These multiple-gene approaches in recent studies will provide a more effective biomarker through a better understanding of the genetic and molecular basis underlying variable drug response among patients.

4.2. Pharmacogenomics of chemotherapeutic toxicity

The variability of pharmacokinetics is caused by the difference in metabolisms and disposition of the drug. Pharmacogenomic studies focusing on the drug metabolizing enzymes and cellular membrane transporters have provided several distinct genotypes relevant to the variable pharmacokinetics and drug toxicity among patients. Individual optimization of gastric cancer chemotherapy based on the contribution of the suggested toxicity biomarkers to the therapy, however, is still restricted to within narrow limits. Very few definitive toxicity markers have been identified to date, except some prominent genotype markers such as *DYPD*2* for 5-FU and *UGT1A1*28* for CPT-11 [8,9,27–30,32–34].

As stated earlier, DPD is the initial rate-limiting enzyme in the degradation of 5-FU and is known to be a principal factor in 5-FU pharmacokinetics, clinical toxicity, and drug resistance [37]. The enzyme demonstrates considerable variation (8- to 21-fold) in both healthy and cancer populations: 3-5% of individuals have reduced DPD activity, which is associated with severe, sometimes life-threatening, 5-FU toxicity among cancer patients [65]. The discovery that DPD deficiency is a pharmacogenetic disorder promoted the discovery of DPD gene (*DPYD*) mutations that are closely linked to DPD toxicity; to date, more than 20 polymorphisms of *DPYD* have been reported. Among these polymorphisms, the exon 14-skipping mutation (*DPYD*2A*) appears to be the most prominent genetic change related to severe DPD deficiency [66,67]. However, these variant alleles are insufficient by themselves to explain either polymorphic DPD activity

in vivo or the majority (>85%) of cases of reduced DPD activity in cancer patients with 5-FU toxicity [68]. Since various reports have clearly demonstrated that DPD activity closely correlates to the mRNA levels, recent attention has been focused on the regulatory mechanisms of *DPYD* expression. Nevertheless, unlike the well-characterized expression profiles of *DPYD* in cancer cells, the regulatory mechanisms of its expression remain unclear [38,39].

UDP-glucuronosyltransferase 1A1 gene polymorphism *28 (*UGT1A1*28*) is a "valid" biomarker of FDA for irinotecan (CPT-11) toxicity as well as of DPD deficiency for 5-FU [23,27–30]. UGT1A1 is the major isoform responsible for the glucuronidation of bilirubin and SN-38, the active metabolite of irinotecan. The enzyme activity varies significantly among individuals, suspecting a 17-fold difference in the rate of SN-38 glucuronidation with *in vitro* observation [69]. The TATA element of the *UGT1A1* promoter region is known to have several variants, with TA repeats ranging from 5 to 8, and having six repeats (TA6) is recognized as the wild type. *UGT1A1*28*, or *7/7*, is the most common variant, having 7 TA dinucleotide repeats, and significantly reduces the gene expression, the enzyme activity, and, therefore, glucuronidation of SN-38, which results in greater tissue exposure to this active metabolite and yields severe CPT-11 toxicity, especially neutropenia [27–30,69,70]. Homozygosity for UGT1A1*28 occurs in 19–24% of the population in the Indian subcontinent, 12–27% of African population, 5–15% of Caucasian population, but only 1.2–5% in South-East Asian and Pacific populations [27–30,34]. Various studies have shown the correlation between *UGT1A1*28* genotype and irinotecan toxicity, but the predictive value of the toxicity remains controversial. A variety of factors have been suggested to be of predictive benefit in CPT-11 toxicity other than *UGT1A1*28*A, such as several membrane transporters, other *UGT1A1* polymorphisms including 211G>A (*6), 1456T>G (*7), 686C>A (*27), another UGT subtype, *UGT1A7*, and, furthermore, several of their combinations, but translating the information to clinical practice is still in the early stages [69].

Pharmacogenomic studies on new oral 5-FU analogs, such as S-1 and capecitabine, and biological agents directed toward identifying the biomarkers of individual response to drugs for both toxicity and effect are currently in progress, along with the clinical development study of a better combination regimen [27–30,66,71].

5. Conclusion

In gastric cancer, radical surgery remains the standard form of therapy with curative intent. Although no global standard regimen has been established to date, active "new generation agents" – taxanes, irinotecan, novel oral fluoropyrimidines and, more recently, biological agents – offer hope for improving patient outcomes. Current chemotherapeutic trials revealed several combinations to be a possible standard treatment, including TCF and cisplatin/S-1. Along with these development studies of novel active regimens, individual optimization of cancer chemotherapy has been attempted in order to reduce toxicity and enhance tumor response. Unlike the rare and limited contribution of pharmacokinetic studies, pharmacogenomic studies are increasing the potential to realize the therapeutics in various malignancies, including gastric cancer. We look forward to more data emerging from ongoing trials. We believe that future large trials would provide the best chemotherapy regimen and the best predictive biomarker for individual toxicity risk and therapeutic benefit in gastric cancer patients.

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Preface

Recent Advances in Cancer Chemotherapy: Current Strategies, Pharmacokinetics, and Pharmacogenomics☆

Despite improvements in the chemotherapeutic treatment of cancer, the existing chemotherapeutic regimens that use classical cytotoxic agents have obvious limitations, including that the narrow therapeutic index does not always allow for the administration of a sufficient amount of the drug in order to induce the intended response. In addition, the high interindividual variability of the drug kinetics makes it difficult to optimize the therapy for each patient [1,2]. Furthermore, the response to chemotherapy varies significantly among individual patients, and numerous patients have been known to continue to undergo a therapeutic regimen without any observable benefits [3]. Therefore, the development of new agents that are capable of far greater specificity in inducing cancer cell death and the implementation of a novel therapeutic strategy that would allow for the selection of an optimal regimen and dose for each individual patient are urgent matters in cancer chemotherapeutics. Interestingly, recent advances in fundamental science and technology have provided opportunities to reexamine and improve the therapeutics involved.

Progress in biomedical technology has rapidly expanded the search-range for potential drug targets and drug response markers from previously limited areas to the whole-genome and whole-gene levels [4]. The increase in our level of understanding of cancer biology has led to various "new generation agents" that include biological agents that are capable of targeting cancer-specific molecules, thus offering hope for improving patient outcomes [5–8]. Pharmacogenomics, a large-scale systematic approach to the genetic basis underlying the variable drug response in individual patients, has provided a variety of potent biomarkers for drug response. Pharmacogenomics has increasingly been recognized as an effective method to optimize the therapy and the treatment dose for each individual [4,9], and accumulating evidence has promoted the clinical application of biomarkers. Furthermore, this genomic research could also have a significant impact on the development of novel drugs. Interestingly, the comparison of normal tissues with cancer tissues, from a more global perspective, may in fact lead to the discovery of novel cancer-specific targets and the development of a more lucid understanding of the inherited causes of severe toxicity and ethnic variability. Such knowledge could decrease the risk of harm or death in clinical trials and in principle reduce the size of studies in different populations. Pharmacogenomics offers a new and exciting dimension to personalized medicine and novel drug development.

It is well known that the variability in pharmacokinetics is a major cause of the differences in the drug response between individual patients. Pharmacogenomics is an area that deals with the genetic basis underlying such variability in drug kinetics, along with the genetic polymorphisms in the intended drug targets [10]. The differences in drug metabolism and in the disposition of drugs have an even greater influence on the toxicity and efficacy of medications, so information regarding the pharmacokinetic properties of each drug is essential in the early stages of drug development and may be of key importance in adjusting treatment doses and schedules for individuals when the initial treatment fails due to excessive toxicity or other complications. Thus, the application of pharmacokinetics, such as in therapeutic drug monitoring, on a routine basis during therapy would have a positive impact on the optimization of chemotherapy [11].

The current era has been widely recognized as an extraordinary period of unprecedented opportunity in cancer drug discovery and in the development of new therapeutics. With that said, the most innovative attempts are still only at the investigational phase, where numerous obstacles still remain [12]. In this issue, four experts review the current strategies regarding chemotherapeutic treatments for colo-rectal, breast, esophageal, and gastric cancers focusing on the most cutting edge pharmacokinetic and pharmacogenomic strategies in clinical development and practical medicine. Numerous reviews are available regarding recent advances in cancer chemotherapy and pharmacogenomics, but only a few of these have focused on the application of pharmacogenomics and pharmacokinetics specifically in the clinical development of a promising new chemotherapy, especially for regimens employing a combination of drugs. These reviews highlight the future directions, possibilities, as well as the limitations of cancer chemotherapy.

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Expert Opinion

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EMP3 as a candidate tumor suppressor gene for solid tumors

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Background: Epithelial membrane protein 3 (*EMP3*), was recently reported to be a tumor suppressor gene for several solid tumors, and is drawing attention as a novel prognostic marker, since its expression level or hypermethylation of the promoter region is associated with clinical prognosis in neuroblastoma and esophageal cancer. However, some controversial data were also observed in gliomas and breast cancers, and there seems to be more than deletion/hypermethylation to its silencing mechanisms. *Objective*: To clarify the discrepancies in the biological behavior of EMP3 among the different organ-derived malignancies or histologies and validate the potential of *EMP3* as a tumor suppressor for solid tumors. *Methods*: Literature dealing with *EMP3* in the PubMed database was reviewed. *Results/conclusions*: *EMP3* is a novel tumor suppressor gene in some kinds of malignancies, but not all, at the step of cellular immortalization rather than carcinogenesis. It may become a potent prognostic marker and a therapeutic target in such tumors.

Keywords: epithelial membrane protein-3 (*EMP3*), esophageal cancer, glioma, methylation, neuroblastoma, prognostic marker, promoter, solid tumor, tumor suppressor gene

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1. Introduction

Recent advances in genome-wide-scale analyses using microarray techniques have made it possible to find novel cancer-associated genes [1,2]. We have also found several genes commonly repressed in esophageal cancer cells by microarray analysis [3]. Interestingly, one of them, the epithelial membrane protein 3 (*EMP3*) gene, was revealed to have been reported as a candidate tumor suppressor gene for neuroblastoma and gliomas [4]. In this review, we summarize the characteristics of *EMP3* as a novel tumor suppressor gene for multiple neoplasms and its regulation mechanisms, and discuss its possible usefulness as a target of anticancer strategies.

2. Biological characteristics of EMP3

2.1 Cloning and mapping of *EMP3* gene

The human *EMP3* (or membrane protein Y (*YMP*)) gene was identified by homology screening in databases as a gene homologous to *EMP1* (or tumor-associated membrane protein (*TMP*)) and peripheral myelin protein 22 (*PMP22*; or growth arrest-specific 3 (*GAS3*)), and was proposed to belong to a family of membrane glycoproteins, PMP22/EMP/membrane protein 20 (MP20) family [5-7]. The *EMP3* gene was mapped to chromosome 19q13.3 in humans (**Table 1**) [8] and chromosome 7 in mice [9]. Human EMP3 consists of 163-amino acids and shares 41, 33, 38, and 23% amino acid identity with PMP22, EMP1, EMP2, and MP20, respectively [6].

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Table 1. Characteristics of PMP22/EMP/MP20 family members [5,6,8,11,15,35-38].

Gene	Alternative name	Gene locus	Expression in tumor	Expression in normal tissue/cell	Genetic aberration	MW (Da)	aa	Homology to PMP22
PMP22 (peripheral myelin protein 22)	GAS3 (growth arrest-specific 3)	17p11.2	Similarly expressed in brain tumor and normal brain	Mainly in peripheral nerve myelin, and also in various adult and fetal tissues. High in growth arrested and low in proliferating NIH-3T3 fibroblasts	Charcot-Marie-Tooth syndrome (duplication, mutation) and other neuropathies	17,891	160	-
EMP1 (epithelial membrane protein 1)	TMP (tumor-associated membrane protein), CL-20, B4B	12p12.3	High in brain tumor	Squamous epithelia and undifferentiated embryonic stem cells but low/none in general. High in proliferating and low in growth arrested NIH-3T3 fibroblasts		17,563	157	40%
EMP2	XMP, MGC9056	16p13.2		Expressed in most tissues, especially in adult ovary, heart, lung, and intestine, and in fetal lung		19,199	167	43%
EMP3	YMP, HNMP-1 (hematopoietic neural membrane protein)	19q13.3	Repressed in some tumors and high in others	Expressed in most tissues, especially in peripheral blood leukocytes, ovary, intestine, and various embryonic tissues	rs4893 variant allele is frequent in prostate cancer	18,429	163	41 – 44%
LIM2 (lens fiber membrane intrinsic protein)	MP18, MP19, (MP20)	19q13.4		Membrane protein of lens fiber	Cataract (F105V mutation)	19,674	173	24%

2.2 Biological characteristics of EMP3

The PMP22/EMP/MP20 family proposed by Taylor *et al.* [7] includes four membrane glycoproteins, PMP22 and EMP1, 2, and 3, that have four transmembrane domains with multiple consensus sequences for N-linked glycosylation in the first hydrophilic domain and a distantly related protein, lens intrinsic membrane protein 2 (LIM2; originally named MP20) [5,10]. *EMP3* mRNA was expressed in all adult and fetal tissues examined with the highest expression in peripheral blood leukocytes and weakest in brain [6]. Although the function of the EMP3 is largely unknown, it was reported that rapid and sustained *EMP3* expression in the peripheral nerve distal to an injury was observed, while *PMP22* expression was rapidly inversely downregulated, indicating an early involvement of EMP3 in Schwann cell proliferation and a sustained role in the regeneration of the nerve [11]. Originally, inverse regulation was reported between PMP22 and EMP1 in this family: the former was apparently downregulated while the latter was highly expressed in proliferating NIH-3T3 fibroblasts [5], and the former was related to apoptosis [12] or function as an adhesion molecule [13] while the latter was related to invasive and metastatic properties of human mammary carcinoma cells [14] as well as squamous differentiation in rabbit tracheal epithelial cells *in vitro* [15]. So, in injured nerves, EMP3 acted like EMP1, and EMPs were predicted to be involved in the regulation of cell-cell interactions and in the control of cellular proliferation [5]. And the fact that its expression levels in fetal brain, lung, liver and kidney were higher than the corresponding counterparts in adults [6] indicated its developmental regulation.

Thus, *EMP3* has been considered to be involved in development and regeneration of nervous system and analogously of hematopoiesis, since aberrations of the most characterized member of this glycoprotein family, *PMP22*, are well known to be responsible for Charcot-Marie-Tooth syndrome (duplication in CMT1A and a sequence variant in CMT1E) and hereditary neuropathy with liability to pressure palsies (deletion or point mutation) (reviewed in [16]). However, it was gradually revealed that the role of EMP3 is not limited in normal nerves or hematopoietic cells only. Rat homolog Emp3 was reported to negatively control dome formation in a rat mammary cell line LA7, which is a manifestation of vectorial transepithelial transport of water and solutes implying differentiation, by inhibiting the expression of Na^+ channel β subunit [17]. Then, DMSO induced dome formation by suppressing *Emp3* expression. Meanwhile, the EMP2 and all other members of the epithelial membrane protein family (EMP1, EMP3 and PMP22) were found to lead HEK-293 cells to cell blebbing, annexin V binding, and cell death, by a caspase-dependent pathway [18]. EMP3 was also reported to play a cytoprotective role in free fatty acid toxicity, possibly by regulating membrane integrity in hepatocarcinoma cell line HepG2 [19]. Recently, reports are focusing on its role in cancer cells as described below.

3. *EMP3* in malignancies and normal tissues

Recently *EMP3* has drawn a lot of attention as a novel and potent tumor suppressor gene in several solid tumors, after the discovery of frequent inactivation of this gene in them [3,4,20]. However, it was originally reported as a candidate for a genes associated with the invasive status in human mammary carcinoma cell lines [21]. Then, the opposite possibility was reported, that *EMP3* was repressed in neuroblastoma and gliomas [4]. Then, bipolar dysregulation was reported in gliomas [20,22], with glioblastomas always upregulated, and in digestive organ-derived cancers [3], commonly repressed in esophageal squamous cell carcinoma (ESCC) cell lines and commonly overexpressed in gastric and colon cancer cell lines. Tables 2 and 3 summarize the *EMP3* expression levels and CpG hypermethylation frequencies in various malignancies reported so far.

3.1 Neuroblastoma

The neuroblastoma cell lines SK-N-BE(2)C, IMR-32, and LAN-1, all having v-myc myelocytomatosis viral related oncogene (*MYCN*) amplification and higher malignant potential belonging to the undifferentiated or neuroblastic cell types, were reported to have *EMP3* promoter hypermethylation and showed no detectable mRNA expression by RT-PCR (33.3% of overall neuroblastoma cell lines and 50% of *MYCN*-amplified neuroblastoma cell lines examined) [4]. This repression was restored after demethylation by 5-aza-2′-deoxycytidine treatment. The remaining six cell lines, including all *MYCN*-non-amplified and/or Schwannian cell type cell lines, had strong *EMP3* expression evaluated by RT-PCR and no evidence of promoter methylation.

For clinical samples, the *EMP3* CpG island hypermethylation evaluated by methylation-specific PCR (MSP) analysis was reported to be found in 24.1% of neuroblastoma tissues (28 of 116) and associated with loss of heterozygosity at 19q13.3, one of the common genetic aberrations in neuroblastoma and gliomas ($p = 0.004$), suggesting a role for *EMP3* as a putative tumor suppressor gene in this locus [4] and that the inactivation mechanisms of *EMP3* in neuroblastoma might be deletion and/or promoter hypermethylation. Furthermore, the *EMP3* CpG island hypermethylation was significantly associated with poor survival in patients that remained alive after 2 years follow-up ($p = 0.030$). Whereas prognostic factors for rapid tumor progression and increased short-term mortality, such as *MYCN* amplification, advanced age or stage, diploidy and high telomerase activity, are well known (reviewed in [23,24]), *EMP3* was proposed to be the first candidates that predicted the late-term prognosis of patients after 2 years survival [4]. Margetts *et al.* recently reported higher methylation frequency of the *EMP3* CpG island in neuroblastomas (68.4%, 13 of 19 cases) [25], possibly due to the difference in MSP sensitivity or cut off value.