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 pharmacodynamic 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 (*CESs*) 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.

Masahiko Nishiyama
Hidetaka Eguchi
(Theme Editors)

Translational Research Center
Saitama Medical University International Medical Center
1397-1 Yamane, Hidaka, Saitama 350-1298, Japan
E-mail address: yamacho@saitama-med.ac.jp

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Expert Opinion

EMP3 as a candidate tumor suppressor gene for solid tumors

Shoichi Fumoto, Keiji Tanimoto, Eiso Hiyama, Tsuyoshi Noguchi, Masahiko Nishiyama & Keiko Hiyama†

†*Hiroshima University, Research Institute for Radiation Biology and Medicine (RIRBM), Department of Translational Cancer Research, Hiroshima, Japan*

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.

Table 2. *EMP3* expression in malignancies.

Tumor tissue				Cell line					Method	Ref.
Neuroblastoma	**Glioma**	**ESCC**	**Breast cancer**	**Neuroblastoma**	**Brain tumor**	**ESCC**	**Breast cancer**	**Lung cancer**		
							None 70% (14*/20)		Real-time RT-PCR	[21]
Ganglioneuroma				None 33% (3‡/9)	None 50% (1§/2)				Cell line: RT-PCR Tissue: microarray	[4]
	OG: low 20% (8/41), high 15% (6/41) OA: low 13% (2/16), high 31% (5/16)								Real-time RT-PCR	[20]
	OG: low 39% (12/31), high 26% (8/31) OA low 40% (4/10), high 40% (4/10) AC: low 21% (4/19), high 42% (8/19) pGBM low 0% (0/9), high 89% (8/9) sGBM low 0% (0/9), high 100% (9/9) pGBM > sGBM, AC								Real-time RT-PCR	[22]
	Glioblastoma: very high								Microarray	[26]
		Generally similar				Low 100% (20/20)	Low 75% (6/8)	Low 11% (1/9)	Real-time RT PCR (compared with normal tissue)	[3]
			Tumor > normal; higher in aggressive type; higher with HER2+						RT-PCR, real-time RT-PCR	[32]

*None: includes all non-invasive type cell lines.

‡None: CpG hypermethylated and *MYCN* amplified cell lines only.

§None: glioma cell line (The remaining was a medulloblastoma cell line).

AC: Astrocytoma and anaplastic astrocytoma; ESCC: Esophageal squamous cell carcinoma; HER2: Human EGF receptor 2; OA: Oligoastrocytoma and anaplastic oligoastrocytoma; OG: Oligodendroglioma and anaplastic oligodendroglioma; pGBM: Primary glioblastomas; sGBM: Secondary glioblastomas.

Table 3. *EMP3* promoter hypermethylation in malignancies.

Tumor tissue					Cell line						5-aza-dC treated cell line		Ref.
Neuroblastoma	Glioma	ESCC	Breast cancer	Phaeochromocytoma	Neuroblastoma	Brain tumor	ESCC	Breast cancer	Lung cancer	Method	EMP3 expression level	Method	
24% (28/116)	39% (16/41)				33% (3*/9)	50% (1‡/2)				MSP	Restored	MSP	[4]
	Oligodendroglial tumor: 90% (26/29)									Bisulfite sequence			[20]
	OG: 76% (44/58) OA 75% (12/16) AC: 84% (41/49) pGBM 17% (5/30) sGBM 89% (8/9)									Bisulfite sequence			[22]
68% (13/19)				6% (2/33)	Very frequent (?/10)					MSP	Up 1.61 – 2.94-fold restored	Microarray	[25]
		6% (1/17)					75% (15/20)	75% (6/8)	22% (2/9)	Quantitative MSP	Up 1.4 – 2.0-fold restored (still low)	Real-time RT PCR	[3]
			36.5% (23/63)							MSP			[32]

**MYCN* amplified cell lines only.

‡Glioma cell line (The remaining line was a medulloblastoma cell line).

AC: Astrocytoma and anaplastic astrocytoma; OA: Oligoastrocytoma and anaplastic oligoastrocytoma; OG: Oligodendroglioma and anaplastic oligodendroglioma; pGBM: Primary glioblastomas; sGBM: Secondary glioblastomas.

These findings indicate that the *EMP3* repression is related to malignant potential and regulated by promoter methylation in neuroblastoma.

3.2 Brain tumor

EMP3 has been reported to be hypermethylated in its promoter region and strongly repressed in a glioma cell line U-87, but not in a medulloblastoma cell line D283 [4].

For clinical samples, the *EMP3* CpG island hypermethylation evaluated by methylation-specific PCR analysis was reported to be found in 39.0% of glioma tissues (16 of 41) and no association was found with age of onset, sex, or histological types [4]. Li *et al.* reported that only 17.5% (10 of 57 cases) of oligodendroglial tumors showed reduced *EMP3* mRNA expression, 6 of them also carried a 19q13 deletion, and 19.3% (11 of 57 cases) showed enhanced expression, while 89.7% (26 of 29 cases examined) showed aberrant methylation of the CpG sites independent of the expression levels, indicating that methylation alone does not mediate transcriptional downregulation of *EMP3* in oligodendroglial tumors [20]. Reduced *EMP3* expression was frequently accompanied with deletion of 1p36 and/or 19q13, while enhanced expression never was.

Kunitz *et al.* reported that 16 of 41 oligodendroglial tumors (39%) but only 4 of 37 astrocytic tumors (11%) exhibited reduced *EMP3* mRNA levels by at least 50% relative to non-neoplastic brain tissue [22]. The *EMP3* expression levels were lower in oligodendroglial tumors with allelic losses on 19q compared with those without losses ($p = 0.01$), but were similar in astrocytic tumors. The *EMP3* mRNA expression was significantly higher in primary glioblastoma multiforme when compared with either secondary glioblastoma multiforme ($p = 0.008$), diffuse astrocytoma WHO grade II (AII) ($p = 0.005$), or anaplastic astrocytoma WHO grade III (AIII) ($p = 0.009$) [22]. Although *EMP3* overexpression in primary glioblastomas compared with non-neoplastic white matter tissue was confirmed also by others [26], there is no information yet on this upregulation of EMP3 expression in primary glioblastoma multiforme. Unexpectedly, it was demonstrated that the *EMP3* hypermethylation was associated with longer overall survival in the 46 patients with oligodendroglial tumors ($p = 0.0323$) by univariable analysis. However, multivariable analysis using Cox's proportional hazards regression model identified 1p/19q loss ($p = 0.04$), but not *EMP3* hypermethylation, as an independent indicator of better prognosis. Thus, the relationship of *EMP3* hypermethylation and favorable prognosis might not be due to the biological consequence of EMP3 inactivation but possibly due to the 1p and 19q losses which are frequently observed in combination and known as a favorable prognostic factor in oligodendrogliomas (reviewed in [27]). It was also reported that compared with benign ganglioneuromas, *EMP3* expression in gliomas was less than one-third [4], while it was significantly higher in glioblastomas [26].

3.3 Esophageal cancer

EMP3 was repressed in all 20 ESCC cell lines examined, 7 with CpG hypermethylation, 8 with partial methylation, and five of them without methylation [3]. Nevertheless, *EMP3* promoter methylation ratios calculated by quantitative MSP using fragment analysis and mRNA expression levels evaluated by real-time RT-PCR in ESCC cell lines were inversely correlated ($r = -0.73$, $p = 0.0002$). The *EMP3* repression was not sufficiently restored after demethylation by 5-azacytidine treatment. These findings indicated that not only CpG island hypermethylation but also other mechanisms must be involved in *EMP3* repression in ESCC [3]. Interestingly, the *EMP3* mRNA expression levels were inversely correlated with telomerase reverse-transcriptase gene (*TERT*) mRNA, in 20 ESCC cell lines ($r = -0.42$, $p = 0.065$) and *EMP3*-transfected clones (two and three clones each in two ESCC cell lines). Telomerase is a well known enzyme that can endow eukaryotic cells with extended lifespan or cellular immortality, by compensating for telomere shortening due to end-replication problems in cell division [28,29].

For clinical samples, the *EMP3* CpG island hypermethylation was partially detected in only one of 17 ESCC tissue samples (5.9%) and all the remaining cases showed no evidence of promoter hypermethylation evaluated by quantitative MSP using fluorescent primers, while mRNA expression level was low in one third cases [3]. Interestingly, in patients with advanced esophageal cancers who had received curative esophagectomy followed by 5 fluorouracil (5-FU)/ *cis*-diamminedichloroplatinum (CDDP) combination chemotherapy, disease specific survival rate after recurrence was significantly poorer in ESCC cases with low *EMP3* expression compared with those with high expression ($p = 0.05$), while disease-free survival (DFS) was similar in the two groups [3]. This finding indicates that suppression of *EMP3* may not provide growth advantage in early stages but works in late stages. This finding seems similar to the prognosis in neuroblastoma, as the promoter hypermethylation in *EMP3* is related to long-term prognosis of patients after 2 years survival [4].

3.4 Breast cancers

EMP3 mRNA was reported to be completely repressed in 14 of 20 breast cancer cell lines, including all 12 non-invasive phenotype (less ability to penetrate into a collagen-fibroblast matrix, many of them with estrogen receptor and progesterone receptor but not plasminogen activator inhibitor-1 expression) [21]. Interestingly, *EMP3* was identified as candidates of invasive type-specifically overexpressed genes with the other members of the transmembrane glycoprotein family, *EMP1* and *PMP22*. We also found the *EMP3* mRNA repression in six of eight breast cancer cell lines (75%), five lines were overlapped with the above, and all these six cell lines had CpG methylation [3]. Thus, *EMP3* repression is probably caused by CpG hypermethylation in almost all the noninvasive phenotype and a part of the invasive phenotype

breast cancer cell lines. In addition, it was reported that *EMP3* expression, as well as that of *EMP1*, was significantly upregulated in *ERBB2* (HER2/*neu*) transfectants of human mammary luminal epithelial cells in an expression-level-dependent manner [30]. Since *ERBB2* overexpression has been reported to be associated with poor prognosis, and resistance to both chemotherapy and endocrine therapy [31], above findings indicate that EMP3 repression might be involved in breast cancers only with low-malignant phenotype, and it may be upregulated in others. Also in clinical samples, Zhou *et al.* reported that the expression levels of *EMP3* mRNA in primary breast carcinomas were significantly higher than those in normal breast tissues ($p < 10^{-7}$), and significantly related to aggressive phenotypes such as histological grade III, lymph node metastasis, and strong human EGF receptor 2 (Her-2) expression [32]. The biological meaning of *EMP3* upregulation is discussed in 'Expert opinion', but remains to be confirmed.

3.5 Other malignancies

We also found *EMP3* mRNA repression in only one of nine lung cancer cell lines (11%) with partial CpG methylation, and the remaining eight lung cancer cell lines and both gastric- and all four colon-cancer-derived cell lines examined showed high expression [3]. These findings indicate that *EMP3* repression in cancers is organ-specific phenomenon, common in esophageal cancer, relatively common in neuroblastoma, glioma and breast cancer, and rare in lung, gastric, and colon cancer cell lines. In other tumor tissues, the hypermethylation of the *EMP3* CpG island in pheochromocytoma was reported to be rare, only in 2 of 33 cases (6.1%), independent of sporadic or VHL-associated cases [25].

3.6 Normal cell/tissues

In all normal tissues analyzed, including lymphocytes, adrenal medulla tissues [4], esophageal tissues, bronchial epithelial cells, mammary cells, fibroblasts [3], and brain tissues [20,22], the *EMP3* CpG island was completely unmethylated without mRNA repression. These findings also support the role of *EMP3* as a tumor suppressor gene.

4. *EMP3* as a tumor suppressor gene

Whereas some controversial findings are observed in breast cancers and gliomas, various pieces of evidence of *EMP3* as a tumor suppressor gene have been accumulated. The reintroduction of *EMP3* into deficient neuroblastoma cell lines induced tumor suppressor-like features, such as reduced colony formation density ($p < 0.001$) *in vitro* and tumor growth ($p < 0.001$) in nude mouse xenograft models [4]. Transfection of *EMP3* into HEK-293 cells, transformed human embryonic kidney cells, induced cell blebbing and annexin V binding resulting in cell death through the caspase-dependent pathway [18]. We also found that transfection of *EMP3* into esophageal squamous cell carcinoma (ESCC) cell lines induced low cloning efficacy and growth inhibition, in proportion to their EMP3 expression levels (Figure 1, A – C) with enhanced contact inhibition and morphological changes (enlarged and flattened) in some population among the small cells retaining original morphology (Figure 1, D) [3].

5. Regulation mechanism of *EMP3* expression

5.1 Methylation of the *EMP3* promoter region

As summarized in Table 3, *EMP3* CpG island hypermethylation is frequently observed in neuroblastoma, gliomas, esophageal squamous cell carcinoma (cell lines), and breast cancer (cell lines), and much less frequently in pheochromocytoma and lung cancer (cell lines), while it has never beeen observed in normal tissues or cells. In oligodendroglial tumors, *EMP3* mRNA levels were significantly lower ($p = 0.006$) and more commonly reduced ($p = 0.03$) in tumors with *EMP3* hypermethylation when compared with tumors without *EMP3* hypermethylation [22]. Similarly, *EMP3* hypermethylation in astrocytic gliomas was significantly associated with lower transcript levels ($p = 0.005$) [22]. We also reported an inverse relationship between promoter methylation ratios and mRNA expression levels in ESCC cell lines [3]. The repressed mRNA levels were restored qualitatively [4] or quantitatively from 1.61- to 2.94-fold [25] in neuroblastoma cell lines and 1.40- and 2.01-fold in ESCC cell lines [3] by 5-azacytidine treatment. So, there is no doubt that methylation of the *EMP3* promoter region is a major mechanism of EMP3 inactivation. However, in ESCC cell lines, *EMP3* mRNA expression was repressed even without promoter methylation and the restoration by 5-azacytidine treatment in hypermethylated cell lines was insufficient [3]. In oligodendroglial tumors, no correlation was observed between transcript level and methylation status in one report [20], whereas it was observed by Kunitz *et al.* [22], indicating that additional *EMP3* silencing mechanisms exist in these tumors, while all *EMP3* repressed samples were accompanied with promoter methylation in breast cancer cell lines [3].

5.2 Deletion/loss of heterozygosity (LOH)

A significant relationship between the 19q deletion/LOH and *EMP3* mRNA repression has been found in oligodendroglial tumors ($p = 0.01$) [22] and one with *EMP3* promoter hypermethylation was observed in oligodendroglial tumors ($p = 0.02 - < 0.001$ according to histology) [22] and neuroblastoma ($p = 0.004$) [4]. In contrast, overexpression of *EMP3* was significantly associated with balanced 19q13 ($p = 0.002$) [20]. Thus, deletion of the *EMP3* locus may be one of the important mechanisms of *EMP3* inactivation.

5.3 *EMP3* genetic variation

In the 132 gliomas, two constitutional single nucleotide substitutions, rs4893 (missense mutation Ile125Val) and rs11671746 (3'UTR) were detected in exon 5 in four patients whereas no tumor-specific mutation was found in

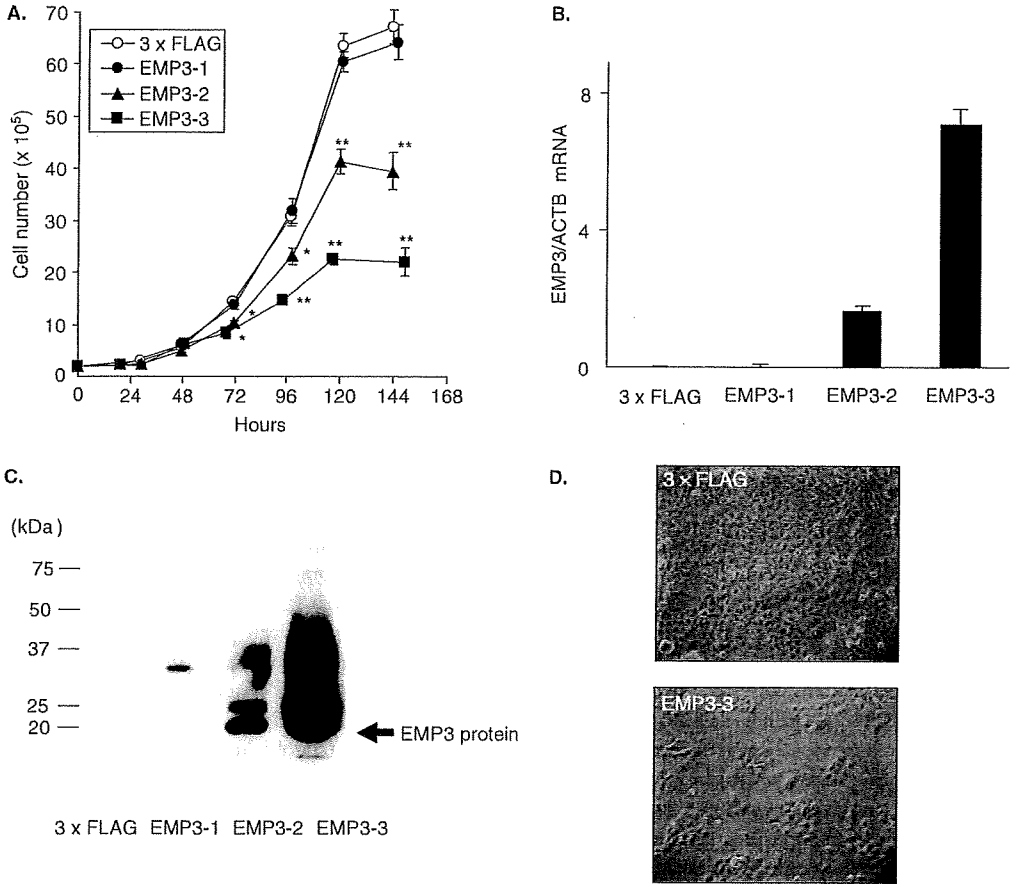

Figure 1. Effects of EMP3 overexpression in ESCC cell line KYSE170 [3]. EMP3 transfected clones EMP3-1, -2, and -3 showed growth inhibition (A) according to their mRNA (B) or protein (C) expression levels, compared to the control clone transfected with the control vector 3 x FLAG. Cell numbers in culture started from 2 x 10^5 in a 10 cm dish are expressed as mean ± SD in triplicate experiments (A). *P < 0.01, **P < 0.001. The *EMP3* mRNA level was evaluated by real-time RT-PCR (B, mean + SD). EMP3 protein amount was evaluated by Western blotting using anti-FLAG M2 as the primary antibody (C, arrow) The EMP3 overexpressing clones EMP3-3 showed morphological changes, being enlarged and flattened, and growth inhibition, compared with the control clone 3 x FLAG (D).

the *EMP3* coding region [22]. The rs4893 variant allele had been reported to be more frequently observed in prostate cancer patients (0.045) than in controls (0.013, $p < 0.0001$) [33], but this low allele frequency indicates that this missense mutation cannot be the major mechanism of EMP3 inactivation. We also investigated the genetic variations in possible promoter regions and the coding regions in ESCC, colon and lung cancer cell lines and tissues principally with low and high *EMP3* expression levels, respectively, and found no tumor-specific mutations that explain the dysregulation of this gene expression [3]. The rs4893 single nucleotide polymorphisms (SNPs) in exon 5 was found only in a lung cancer cell line A549 among 46 individuals examined. We also found a Japanese- or Asian-population-specific haplotype of three SNPs (rs8102349, rs8355 and rs11665, Figure 2) in non-coding regions or introns with variant allele frequency 0.28, but no association was found with the *EMP3* expression levels [3]. Thus, genetic variation is unlikely to contribute to *EMP3* inactivation.

5.4 Unidentified repressor

We found that 24-h treatment with trichostatin A (TSA), which inhibits histone deacetylase (HDAC), induced dramatic repression of the *EMP3* mRNA expression in a dose-dependent manner in all HEEC-1 non-cancerous esophageal epithelial cells and ST11 and TF-1 *in vitro* immortalized fibroblast cell lines that originally had no repression. Meanwhile, no effect was observed in originally *EMP3*-repressed ESCC cell lines and a hepatocarcinoma cell line HepG2 regardless of the promoter hypermethylation status [3]. These findings indicate that there may be a repressor of *EMP3* that is regulated by HDAC in EMP3 expressing cells but active

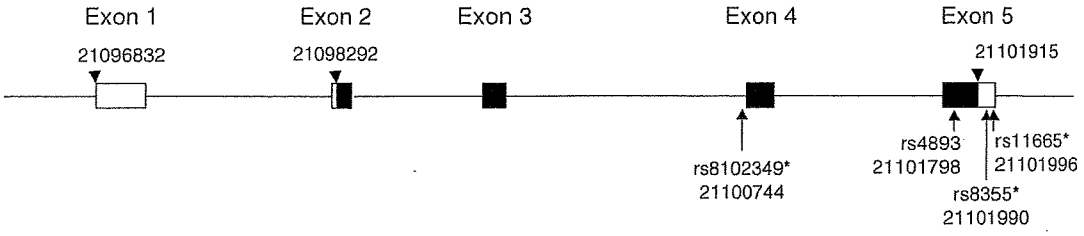

Figure 2. Representative polymorphisms in the *EMP3* gene. Locations of initiation sites of transcription and translation, stop codons (arrowheads), and representative polymorphisms (arrows) are numbered according to the contig positions and rs numbers are cluster IDs registered in dbSNP [35]. Closed box: coding exon, Open boxes: untranslated exon.
*Haplotype found in Japanese [3].

in EMP3-repressed cells without promoter hypermethylation such as ESCC cells. However, the existence of this repressor remains to be elucidated.

6. Prognostic marker

In neuroblastoma, it was reported that *EMP3* is a good candidate for being the long-sought tumor suppressor gene because *EMP3* promoter hypermethylation was significantly associated with poor survival after the first 2 years of onset of the disease ($p = 0.05$) and death from disease ($p = 0.03$) [4]. Similarly, when the ESCC patient prognosis was compared with the *EMP3* expression level, low *EMP3* expression was associated with poor prognosis after recurrence ($p = 0.05$), whereas it was not associated with DFS [3]. However, in oligodendroglial tumors, the *EMP3* hypermethylation as well as the 1p/19q losses were favorable prognosis factors [22]. Further, the most malignant phenotype of gliomas, glioblastoma multiforme, were revealed to have enhanced expression of *EMP3* in all samples examined. In addition, Zhou *et al.* recently reported that *EMP3* overexpression in breast carcinomas was correlated with carcinoma aggressiveness [32]. To explain these discrepancies, we considered that there might be an EMP3-independent factor(s) that promote cellular immortalization in particular malignancies, as proposed in Figure 3 and discussed below.

7. Expert opinion

EMP3, located on chromosome 19q13 [8], has been implicated as a candidate tumor suppressor gene for some solid tumors such as neuroblastoma, glioma and ESCC, with reliable studies using *EMP3* overexpression and knockdown experiments [3,4]. Furthermore, the high frequency of CpG island methylation in ESCC cell lines but not in ESCC tissues, inverse relationship between *EMP3* and *TERT* expression levels in ESCC cell lines, ESCC tissues and *EMP3* transfectants, positive relationship of *EMP3* repression with poor prognosis after recurrence (disease-specific survival (DSS)-DFS) but not with that until recurrence (DFS) in ESCC patients [3], and positive relationship of *EMP3* promoter methylation with poor prognosis after 2-year survival but not in overall prognosis of patients with neuroblastoma [4] indicated that the inactivation of *EMP3* may promote cellular immortalization with telomerase activation in such cancer cells. Tumor tissues may contain certain amounts of mortal cancer cells without telomerase expression while all cells in cell lines are immortal [34], this would explain the difference of *EMP3* methylation frequency between the ESCC cell lines and tissues. Although the tissue heterogeneity of clinical samples may have contributed to a part of this difference, the findings that no tissue sample showed high methylation rate nor absence of expression indicate that it may have derived from the biological difference between tissue samples and cell lines, that is existence of mortal cells or not. Before recurrence, cancer cells have not experienced so many cell divisions to critically shorten their telomeres, explaining the absence of effect of cellular immortalization on DFS, but after recurrence only immortalized cancer cells with telomerase activation could continue to proliferate resulting in short DSS-DFS. However, we speculate that there might be an EMP3-independent factor(s), possibly stronger than EMP3, that promote cellular immortalization in particular malignancies, for example glioblastoma multiforme, aggressive type oligodendroglial tumors, gastric cancer, colon cancer, lung cancer (majority), and invasive phenotype mammary carcinoma cell lines, but not in neuroblastoma and ESCC. If this putative factor exists in a tumor, EMP3 inactivation does not endow these cells with a growth advantage any more, then no promoter methylation nor repression may occur, and on the contrary as an innate inhibitory mechanism of cellular immortalization, *EMP3* might be overexpressed in such tumors (Figure 3). If such an EMP3-independent immortalization promoting factor is the dominant adverse prognostic event, then *EMP3* promoter methylation or 1p/19q losses may correlate with good prognosis in gliomas [22], and *EMP3* expression may be enhanced only in invasive-type breast cancer cell lines and upregulated in *ERBB2* (HER2/*neu*) transfectants of human mammary luminal epithelial cells [30]. In fact, *EMP3* expression levels in almost all of ESCC (20/20) and many breast cancer (20/28) cell lines

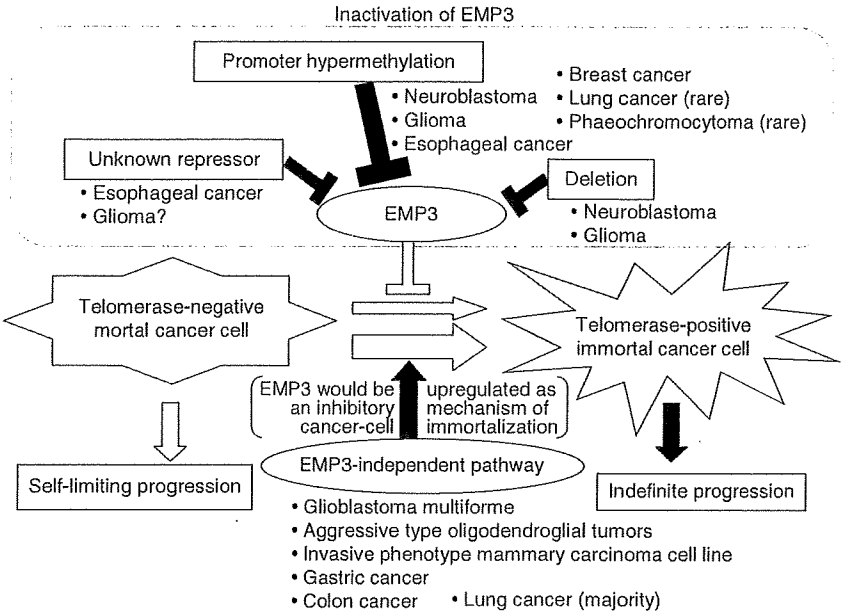

Figure 3. Hypothetical regulation and function of EMP3 in human malignancies (authors' speculation). EMP3 may have an inhibitory role in cellular immortalization of neuroblastoma, glioma, and esophageal cancers, while some EMP3-independent pathway(s) might exist in glioblastoma, aggressive type oligodendroglioma, invasive phenotype mammary carcinoma, gastric cancer, and colon cancer.

were lower than those of non-cancerous strains, but were only in a few lung cancer cell lines (1/9) [3,4]. Meanwhile in tissue samples, *EMP3* expression levels in glioblastoma and breast cancer tissues are higher than those of non-cancerous tissues, but not in ESCC or neuroblastoma [3,4,26,32]. These findings can be explained by our above hypothesis that EMP3 acts as a tumor suppressor gene in various tumors at the cellular immortalization step, but there may be another EMP3-independent pathway for cellular immortalization in particular malignancies, for example, glioblastoma multiforme and breast cancer, but not in ESCC and neuroblastoma (Figure 3).

In conclusion, EMP3 may act as a tumor suppressor in some kinds of malignancies, neuroblastoma and ESCC but not all, at the step of cellular immortalization rather than the step of carcinogenesis. As for the regulation mechanism of *EMP3* expression, allele loss and/or promoter hypermethylation are relatively common, but also an unidentified repressor(s) that is regulated by HDAC may exist at least in ESCC. Genetic mutation is unlikely to be the major mechanism of EMP3 inactivation. Although there are black boxes to be elucidated, *EMP3* can be a potent prognostic marker for some types of solid tumors and allow us to develop a novel molecular targeting therapy in future.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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Affiliation

Shoichi Fumoto[1,5] MD PhD,
Keiji Tanimoto[1] DDS PhD,
Eiso Hiyama[2] MD PhD,
Tsuyoshi Noguchi[3] MD PhD,
Masahiko Nishiyama[1,4] MD PhD &
Keiko Hiyama[†1] MD PhD
[†]Author for correspondence
[1]Hiroshima University,
Research Institute for Radiation Biology and Medicine (RIRBM),
Department of Translational Cancer Research,
Hiroshima, 734-8551, Japan
Tel: +81 82 257 5841; Fax: +81 82 256 7105;
E mail: khiyama@hiroshima-u.ac.jp
[2]Hiroshima University,
Natural Science Center for Basic Research and Development (N-BARD),
Hiroshima, 734-8551, Japan
[3]Faculty of Medicine
Oita University,
Department of Gastrointestinal Surgery,
Oita, 879-5593, Japan
[4]Saitama Medical University
International Medical Center,
Saitama, 350-1298, Japan
[5]Hiroshima University,
Graduate School of Biomedical Sciences,
Division of Clinical Oncology,
Hiroshima, 734-8551, Japan

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Review article

Docetaxel: its role in current and future treatments for advanced gastric cancer

Masahiko Nishiyama[1,2] and Satoru Wada[1,2]

[1]Translational Research Center, Saitama Medical University International Medical Center, 1397-1 Yamane, Hidaka, Saitama 350-1298, Japan
[2]Project Research Division, Research Center for Genomic Medicine, Saitama Medical University, Hidaka, Japan

Abstract
A globally accepted standard chemotherapy remains undetermined in gastric cancer, but the recent introduction of active "new-generation agents" such as taxanes, irinotecan (CPT-11), oxaliplatin, S-1, and capecitabine, offers hope for markedly improving patient outcomes. Docetaxel, as well as the other new-generation agents, plays a key role in the development of the new-era chemotherapy, and the incorporation of taxanes has provided several regimens, such as docetaxel/cisplatin/5-fluorouracil (5-FU) (DCF), that could become standard treatment. The DCF regimen is now regarded as a standard treatment option in advanced gastric cancer in selected patients in good condition. Many institutions and cooperative groups continue to study a variety of docetaxel-based combinations with "new-generation cytotoxic agents" in various treatment settings, and recent attention has been focused on the incorporation of biological agents, such as cetuximab, bevacizumab, everolimus, and sunitinib, into docetaxel-containing combinations as another innovative approach. The ongoing clinical trials of a number of new regimens will clarify their clinical benefits in gastric cancer treatment. Along with the development of more active docetaxel combination regimens, the identification of predictive biomarkers for each regimen has been intensively studied recently. This review focuses on docetaxel as a key agent in gastric cancer chemotherapy, and discusses the role of this taxane in current and future treatments for advanced gastric cancer.

Key words Docetaxel · Gastric cancer · Chemotherapy

Introduction

In gastric cancer, a series of trials have produced evidence that chemotherapy increases survival, but a globally accepted standard chemotherapy and the optimal regimen are undetermined [1–4]. Both CF (cisplatin [CDDP]/5-fluorouracil [5-FU]) and ECF (epirubicin/CDDP/5-FU) have been considered as reference regimens to date [5–8], but the median survival time (MST) of the regimens does not exceed 7–10 months.

Recently, several active agents have been used in gastric cancer therapy: the taxanes, irinotecan (CPT-11), oxaliplatin, S-1, and capecitabine, and more recently, biological agents such as cetuximab and bevacizumab have been used [1–8]. Current studies have thus focused on "new-generation agents," and much effort has been directed towards the development of the best regimen in various treatment settings. A series of these trials have provided several regimens that could become a standard treatment: the regimens include docetaxel/CDDP/5-FU (DCF) and CDDP/S-1 for advanced and metastatic cancer, and S-1 monotherapy in the adjuvant setting [9–11]. The advent of these new-generation agents offers hope for improving patient outcomes.

Among the new-generation agents, docetaxel now appears to be one of the most extensively investigated [12]. Docetaxel has demonstrated promising activity in gastric cancer, both as monotherapy [13, 14] and in combination with other agents [15–17]. To date, docetaxel combinations, especially the DCF regimen, seem to be pivotal [9, 10], and further, a taxane has been suggested to be the best potential partner of new oral 5-FU analogues and prodrugs such as S-1 and capecitabine [16, 17]. This review will focus on the taxane, docetaxel, as a key agent in gastric cancer chemotherapy.

Docetaxel as a single agent

Mechanisms of action and metabolism

Docetaxel is a semisynthetic analogue of paclitaxel, an extract from the rare Pacific yew tree *Taxus brevifolia* [18]. The chemical structure of docetaxel differs from

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that of paclitaxel at two positions, a tert-butyl carbamate ester on the phenylpropionate side chain and a hydroxyl functional group on carbon 10, which causes docetaxel to be more water-soluble than paclitaxel. Docetaxel, a second-generation taxane, binds to and stabilizes tubulin, which prevents physiological microtubule depolymerization/disassembly and results in cell-cycle arrest at the G2/M phase and cell death [19, 20]. This agent also is known to inhibit the anti-apoptotic gene *Bcl2* and to encourage the expression of *p27*, a cell-cycle inhibitor, and further pro-angiogenic factors such as vascular endothelial growth factor (VEGF) [21]. Docetaxel is mainly metabolized in the liver by the cytochrome P450 CYP3A4 and CYP3A5 subfamilies of isoenzymes [18, 22], and its clearance has been shown to be related to body surface area and hepatic enzyme and alpha1 acid glycoprotein plasma levels [23].

Clinical activity and toxicity

A series of phase II trials have shown that docetaxel monotherapy has appreciable activity in gastric cancer. Administration of docetaxel was commonly repeated every 3 weeks at a dose of 60–100 mg/m^2, and the overall response rate (ORR) in the front-line setting was 17%–24%, while in the second-line setting the ORR was 4.8%–22% [12, 13, 24–28]. In chemotherapy-naïve patients, docetaxel monotherapy has achieved ORRs of 18%, 20%, and 24% when given at 100 mg/m^2, and 18% when used at the slightly lower dose of 75 mg/m^2 [13, 25, 27, 28]. In the salvage setting, docetaxel single-agent therapy achieved an RR of 20% when given at 100 mg/m^2, and 22% when used at the dose of 60 mg/m^2 [29, 30].

The most common adverse reactions are infections, neutropenia, anemia, febrile neutropenia, hypersensitivity, thrombocytopenia, neuropathy, dysgeusia, dyspnea, constipation, anorexia, nail disorders, fluid retention, asthenia, pain, nausea, diarrhea, vomiting, mucositis, alopecia, skin reactions, and myalgia [12, 31]. Reversible bone marrow suppression was the major dose-limiting toxicity in patients with various tumor types receiving docetaxel monotherapy at 100 mg/m^2 [32]; the incidence of neutropenia was 95.5%; anemia, 90.4%; febrile neutropenia, 11.0%; and thrombocytopenia, 8.0% [31]. At least 95% of these patients, however, recovered without receiving hematopoietic support. In the 40 phase II and phase III studies, deaths due to toxicity accounted for 1.7% of the 2045 patients and the incidence of such deaths was increased (9.8%) in patients with elevated baseline liver function test results (liver dysfunction).

Docetaxel-containing combinations

The early studies of docetaxel monotherapy indicated that docetaxel was well tolerated, active in advanced gastric cancer, and deserved further investigation in multidrug combination programs.

Combinations with "classical" cytotoxic drugs

For advanced, metastatic, and recurrent gastric cancer, 5-FU- and/or CDDP-based combinations are still the mainstay of treatment [1–8, 33–44] (Table 1). Both CF (CDDP/5-FU) and ECF have been recognized as the most active treatment options in various countries, especially in the United States and Europe. The MST, however, does not exceed 7–10 months with these "classical" combinations. On the basis of the encouraging results observed in monotherapy, combinations of docetaxel with the "classical" regimens or drugs have been intensively investigated. These studies have developed several active regimens including a pivotal triplet regimen, DCF (docetaxel/CDDP/5-FU), which has been approved as a treatment for advanced gastric and gastroesophageal adenocarcinoma by the Food and Drug Administration of the United States based on the results of the V-325 study group.

The V-325 study group first investigated the DCF triplet regimen (docetaxel [75 mg/m^2], CDDP [75 mg/m^2], and 5-FU [750 mg/m^2/day × 5 days] every 3 weeks) and a doublet regimen (docetaxel [85 mg/m^2] and CDDP [75 mg/m^2] every 3 weeks) in a randomized phase II study [45]. This trial demonstrated that the triplet regimen (n = 79) was superior to the doublet one (n = 76) in terms of ORR (43% vs 26%), and time to progression (TTP; 5.9 months vs 5.0 months) in patients with metastatic gastric cancer. DCF was chosen as the investigational regimen based on the higher ORR and acceptable toxicity profile. The following multinational phase III trial with the endpoint of TTP enrolled and randomized 455 patients across 72 centers and 16 countries with DCF (n = 221) and CF (n = 224). The trial demonstrated that the DCF triplet regimen was superior to CF in terms of ORR (37% vs 25%; 95% confidence interval [CI], 30.3–43.4 vs 19.9–31.7; χ^2; P = 0.0106), TTP (5.6 months vs 3.7 months; hazard ratio [HR], 1.47; 95% CI, 1.19–1.82; risk reduction 32%; log-rank P = 0.0004), and overall survival [OS] (9.2 months vs 8.6 months; risk reduction 23%; HR, 1.29; 95% CI, 1.0–1.6; log-rank P = 0.0201) in patients with metastatic gastric cancer [9, 10]. The 1-year survival rates of DCF and CF were 40% and 32%, respectively, and the 2-year survival rate was doubled with the DCF regimen as compared to CF (18% vs 9%). However, DCF was more toxic than DC – grade III/IV neutropenia (82% vs 57%), leucopenia (65% vs 31%), febrile neutrope-