Acta Oncologica, 2014; 53: 885–891

informa
healthcare

ORIGINAL ARTICLE

The expression of CYP2W1 in colorectal primary tumors, corresponding lymph node metastases and liver metastases

KRISTINA STENSTEDT[1,2], MARJA HALLSTROM[3], FRIDA LÉDEL[1,2], PETER RAGNHAMMAR[3], MAGNUS INGELMAN-SUNDBERG[4], INGER JOHANSSON[4] & DAVID EDLER[1,2]

[1]*Center of Surgical Gastroenterology, Karolinska University Hospital, Stockholm, Sweden,* [2]*Institution of Molecular Medicine and Surgery, Department of Surgery, Karolinska Institutet, Stockholm, Sweden,* [3]*Institution of Oncology and Pathology, Karolinska Institutet, Stockholm, Sweden and* [4]*Institution of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden*

ABSTRACT

Introduction. Metastatic disease is a major cause of death in patients with colorectal cancer (CRC). We have previously investigated expression of an orphan cytochrome P450 (CYP) enzyme, CYP2W1, and found high expression in about one third of colorectal tumors. CYP2W1 has proven to metabolize duocarmycin analogs into cytotoxic substances, compounds that in xenografts of CRC cells expressing CYP2W1 completely inhibit tumor growth. This study was designed to evaluate whether the enzyme is expressed in primary CRC and corresponding metastases.

Material and methods. Samples from primary tumors, corresponding lymph node metastases and liver metastases from 96 patients were collected and analyzed by immunohistochemistry. Data regarding patient's demographics, tumor characteristics and survival were also collected.

Results. Out of 96 patients, 25 (26%) had high CYP2W1 expression in the primary tumor and 46 (48%) showed high levels in the liver metastasis. In total 59 patients had lymph node metastases, and 31% of them had high CYP2W1 expression. When comparing the expression in primary tumor with that of the first liver metastasis, the increase in expression was statistically significant ($p = 0.005$).

Conclusion. High CYP2W1 expression is seen in 26% of primary CRC and in 48% of corresponding liver metastases. This opens possibilities for new targeted therapies to metastatic CRC in the future.

Colorectal cancer (CRC) is the third most common cancer in the world and a major cause of cancer-related death in both men and women [1]. In total 15–20% of CRC patients present with liver metastases at the time of diagnosis and approximately another 20–40% will develop hepatic metastases during the course of disease [2,3]. Survival in CRC is highly stage dependent where stage I have a five-year survival of 90–100%, stage II of 75–85% and stage III 45–60%. Patients with stage IV CRC have a very poor prognosis with five-year survival rates less than 5% [3–8]. Survival in metastatic CRC has improved, though, during the last decade, mainly due to more potent chemotherapy regimens and improved surgical techniques [9]. Hepatic resections improve outcome significantly in patients with colorectal liver metastases. About 20% of metastatic patients are believed to be suitable for liver resection. Population-based studies have shown five-year survival rates between 45% and 50% in liver-resected metastatic patients [9–12]. Still, there is an urgent need to find other ways of treating metastatic CRC, complementary to the established treatments.

We have previously studied tumor-specific expression of an orphan cytochrome P-450 enzyme, CYP2W1, in CRC [13,14]. This enzyme is shown not to be expressed in any normal adult tissue in humans. As far as have been investigated, expression has only been detected in fetal rat colon and

Correspondence: Kristina Stenstedt, Centre for Surgical Gastroenterology, P9:03, Karolinska University Hospital, 17176 Stockholm, Sweden. Tel: +46851779214. Fax: +468331587. E-mail: kristina.stenstedt@ki.se

(Received 7 October 2013; accepted 18 January 2014)

ISSN 0284-186X print/ISSN 1651-226X online © 2014 Informa Healthcare
DOI: 10.3109/0284186X.2014.887224

in various human tumors, most prominently in colorectal tumor specimen [15]. In our previous work, high expression of CYP2W1 correlated independently to poor survival in CRC [13,14] thus indicating that high expression is seen in a more malignant tumor phenotype. The endogenous function of CYP2W1 is unknown, but some fairly recent studies have found a number of substrates metabolized by CYP2W1 although with rather low affinity, e.g. benzphetamine [16], indole derivatives [16,17], the drug candidate 1,4-bis([2-(dimethylamino-N-oxide)ethyl]amino)-5,8-dihydroxyanthracene-9, 10-dione (AQ4N) [18] and arachidonic acid [15]. A lipidomic examination of colon tumors revealed that lysophospholipids were substrates for CYP2W1 [19]. We have recently found that certain duocarmycin analogs act as efficient CYP2W1 substrates in the range of 0.5–2 μM concentrations [20]. Experiments in CRC cell lines (SW480) expressing CYP2W1 have revealed that two of these compounds, ICT2705 and ICT 2706, after conversion by CYP2W1 will bind covalently to DNA causing cell death. The compounds themselves cause no harm to the tumor cells [20]. Xenograft studies, where SCID mice were grafted with CYP2W1 expressing CRC cells and then treated with ICT 2706, have shown promising results with complete inhibition of tumor growth but no apparent harm to the animal [20]. The perspective of finding a tumor-specific enzyme in primary CRC and in metastases with capacity to metabolize a non-toxic substance into a toxic metabolite is encouraging since this might be another plausible pathway to use in cancer treatment.

Since the primary target for a CYP2W1 dependent drug treatment would be metastases originating from the primary tumors, we considered it of interest to examine the expression of CYP2W1 both in primary CRC, corresponding metastatic lymph nodes and liver metastases using the same methods as in our previous studies [13,14].

Material and methods

Patients

From a population-based cohort of 255 patients having undergone liver resection due to colorectal liver metastases at the Department of Hepatobiliary Surgery, Karolinska University Hospital, between 2004 and 2009, we could obtain paraffin-embedded blocks from primary colorectal tumors, corresponding lymph node metastases and liver metastases of 96 patients. Six of the 96 patients had also had a pulmonary resection due to lung metastases, these were also collected and analyzed. No pre-treatment biopsies were available for analysis.

Data from the patient's records regarding gender, age, tumor location, TNM stage, number of lymph nodes sampled at primary surgery, neoadjuvant treatment, synchronous or metachronous metastases, dates for all surgical procedures and survival data were collected. Details about tumor histopathology were derived from the original pathology reports. The primary tumors were operated between 1999 and 2009, and the corresponding liver metastases were resected between 2004 and 2009. We defined metastases diagnosed up to six months after surgery of the primary tumor as synchronous metastases. The Board of Research Ethics at Karolinska University Hospital has approved of the study.

Immunohistochemistry

The examined tumor specimens were derived from formalin-fixated, paraffin-embedded tumors, sliced in 4-μm thick sections. The sections were deparaffinized in xylene, rehydrated in graded ethanol and washed in distilled water. To quench the endogenous peroxidase activity, 3% hydro peroxidase in tap water was added. In order to reduce background staining, the slices were incubated in 10% normal goat serum for 30 minutes. A polyclonal rabbit antibody to CYP2W1 was added in a dilution of 1:2500 at + 4°C overnight. The samples were then rinsed and incubated with an amplification system with a labeled polymer/HRP, EnVision™ (DakoCytomation, Denmark) for 30 minutes. Visualization of staining with 3,3′-diaminobenzidine tetrahydrochloride (DAB, DakoCytomanion) was carried out followed by counterstaining with Mayers hematoxylin.

Evaluation of immunohistochemistry

In most cases, two slides from each tumor manifestation were available for analysis. CYP2W1 staining intensity was defined by a visual grading scale from 0 to 3 (grade 0 = no staining, grade 1 = weak, grade 2 = moderate, grade 3 = intense staining). Each time a set of tumor samples was stained, reference slices were included as well as one negative control slice incubated with pre-immune serum. The whole tumor slide was graded. The grading was based on the highest intensity found in the tumor that covered at least 10% of the tumor area. Two independent investigators (K.S. and M.H.) blinded to clinical data scored the specimens. Scoring discrepancies occurred in less than 5% of scorings and were resolved by consensus after re-examination. In the calculations, we regarded staining degrees 0, 1 and 2 as low, and 3 as high expression like in our previous studies [13,14].

Statistics

χ^2-test was performed to examine relationships between patient's demographics, tumor characteristics and CYP2W1 expression, and when appropriate, Fisher's exact test was used. Survival analysis was performed using the Kaplan-Meier method. Univariate and multivariate analysis were performed using Cox's univariate test and Cox's Proportional Hazards model, respectively. The results were considered significant if $p < 0.05$. All calculations were performed using Statistica version 10 (StatSoft, Tulsa, Oklahoma, USA).

Results

Follow-up

All 96 patients had undergone surgery for the primary tumor and at least one liver resection. In total, 27 had a second operation due to metastases: 20 had liver resections, six lung resections and one had surgery for local recurrence. We focused on the calculations based on the primary tumor and the first liver operation due to the small amounts of patients in the other groups. Survival data was calculated in months after the resection of the primary tumor.

Median follow-up time after the first liver operation was 45.5 months (range 4–110 months). Survival data for the 96 patients are shown in Figure 1. There was no association between survival and CYP2W1 expression, neither in the primary tumor, nor in the liver metastases.

CYP2W1 expression

High CYP2W1 expression was detected in 25 (26%) of the primary tumors. Of the 96 patients, 59 had nodal metastases, and 18 (31%) of these metastases expressed high levels of CYP2W1. In the liver metastases (n = 96), we found high enzyme expression in 46 (48%). Expression levels did not correlate to site of primary tumor, age, gender, stage, differentiation grade, number of nodes analyzed, synchronous versus metachronous liver metastasis, neoadjuvant treatment before primary surgery, adjuvant treatment after primary surgery or chemotherapy before liver surgery. The results are displayed in Table I.

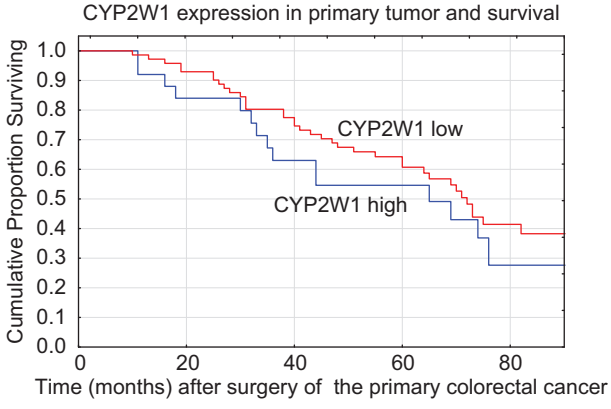

Figure 1. Survival in months after primary surgery in the group with low (group 1) versus high (group 2) expression of CYP2W1. HR 1.3, p = 0.38, 95% CI 0.72–2.38.

In the 20 patients being resected a second time for liver metastases, 10 had high CYP2W1 expression, nine had low expression and one had no remaining tumor in the specimen. The lung metastases (n = 6) showed high expression of CYP2W1 in two cases, the remaining had low expression. All lung metastases had the same degree of expression as their corresponding primary tumor and liver metastasis.

The number of liver metastases with high expression of CYP2W1 was significantly higher than the primary tumors, 46 (48%) versus 25 (26%), p = 0.005. The details about enzyme expression in various locations are shown in Table II and the dynamics of expression are displayed in Figure 2. In the 71 patients with low CYP2W1 expression in the primary tumor, 43 (61%) had low expression also in the metastasis and 28 (39%) had high expression in the liver metastasis. In the 25 patients with high expression in the primary tumor, 18 (72%) remained high in the metastasis while seven (28%) decreased the expression in the metastasis. Separate analysis of the patients with increasing or decreasing CYP2W1 expression during the primary tumor – metastasis progression, revealed no correlation to gender, stage, chemotherapy or any other factor listed in Table I (data not shown).

The concordance of CYP2W1 expression between different slices of the primary tumor was good which we also have previously reported in another study [14].

In the group of patients with metachronous liver metastases, the time span from diagnosis of the primary tumor to that of the liver metastasis was 7–62 months, median 23 months. Six of the 35 patients with metachronous metastases had high CYP2W1 expression in the primary tumor and 29 (83%) had low expression. In the group with low CYP2W1 expression, 15 had their metastasis detected during the time span below median time while 14 were detected during the time span above median. For the high expression group of six patients, five of them recurred in the shorter time span. This difference, though, is not statistically significant (p = 0.21).

Table I. CYP2W1 expression in primary colorectal tumors and liver metastases in relation to patient and tumor characteristics. Definition of synchronous metastasis is detection within 6 months after the detection of the primary tumor.

	Number of patients	CYP2W1 expression Primary tumor, low vs. high	χ^2 p-value	CYP2W1 expression Liver metastases, low vs. high	χ^2 p-value
Total	96	71 (74%) vs. 25 (26%)		50 (52%) vs. 46 (48%)	
Gender			0.86		0.26
Male	59 (61%)	44 (74%) vs. 15 (26%)		28 (47%) vs. 31 (53%)	
Female	37 (39%)	27 (73%) vs. 10 (27%)		22 (59%) vs. 15 (41%)	
Age			0.62		0.23
<63	42 (44%)	30 (71%) vs. 12 (29%)		19 (45%) vs. 23 (55%)	
≥ 63	54 (56%)	41 (76%) vs. 13 (24%)		31 (57%) vs. 23 (43%)	
Stage			0.52		0.38
II	15 (16%)	12 (80%) vs. 3 (20%)		7 (47%) vs. 8 (53%)	
III	25 (26%)	20 (80%) vs. 5 (20%)		15 (60%) vs. 9 (40%)	
IV	56 (58%)	39 (70%) vs. 17 (30%)		27 (48%) vs. 29 (52%)	
Tumor site			0.90		0.29
Colon	51 (53%)	38 (75%) vs. 13 (25%)		24 (47%) vs. 27 (53%)	
Rectum	45 (47%)	33 (73%) vs. 12 (27%)		26 (58%) vs. 19 (42%)	
Differentiation			0.74		0.59
Poor	18 (19%)	12 (67%) vs. 6 (33%)		10 (55%) vs. 8 (45%)	
Moderate	74 (77%)	56 (76%) vs. 18 (24%)		37 (50%) vs. 37 (50%)	
Well	4 (4%)	3 (75%) vs. 1 (25%)		3 (75%) vs. 1 (25%)	
Nodes analyzed			0.62		0.38
<13	42 (44%)	30 (71%) vs. 12 (29%)		24 (57%) vs. 18 (43%)	
≥ 13	54 (56%)	41 (76%) vs. 13 (24%)		26 (48%) vs. 28 (52%)	
Range 3–50					
Neoadjuvant before primary operation			0.91		0.31
No	47 (49%)	35 (74%) vs. 12 (26%)		22 (47%) vs. 25 (53%)	
Yes	49 (51%)	36 (73%) vs. 13 (27%)		28 (57%) vs. 21 (43%)	
Type of neoadjuvant			0.11		0.08
None	47 (49%)	35 (74%) vs. 12 (26%)		22 (47%) vs. 25 (53%)	
Radiation	27 (28%)	23 (85%) vs. 4 (15%)		19 (70%) vs. 8 (30%)	
Radiochemo	11 (11.5%)	5 (45%) vs. 6 (54%)		3 (28%) vs. 8 (72%)	
Chemo	11 (11.5%)	8 (72%) vs. 3 (28%)		6 (54%) vs. 5 (45%)	
Liver metastases			0.13		0.11
Synchronous (≤ 6 months)	61 (64%)	42 (69%) vs. 19 (31%)		28 (46%) vs. 33 (54%)	
Metachronous (>6 months)	35 (36%)	29 (83%) vs. 6 (17%)		22 (63%) vs. 13 (37%)	
Chemo before liver operation					0.06
No	41 (43%)			26 (63%) vs. 15 (37%)	
Yes	55 (57%)			24 (44%) vs. 31 (56%)	

Table II. Distribution of CYP2W1 expression in 96 colorectal cancer patients with liver metastasis.

Localization of high CYP2W1 expression	Number of patients Total n = 96	%
In primary tumor only	3	3.1
In primary + nodal metastasis	4	4.2
In primary + liver metastasis	12	12.5
In primary + nodes + liver	6	6.3
In nodal metastasis only	3	3.1
In nodes + liver metastasis	5	5.2
In liver metastasis only	23	24.0
Σ In primary tumor	25	26.0
Σ In nodal metastasis	18/59	18.8*
Σ In liver metastasis	46	47.9
No/low expression in any location	40	41.7

*Only 59 of the patients had nodal metastasis, i.e. 31% of nodal metastasis expressed high levels of CYP2W1.

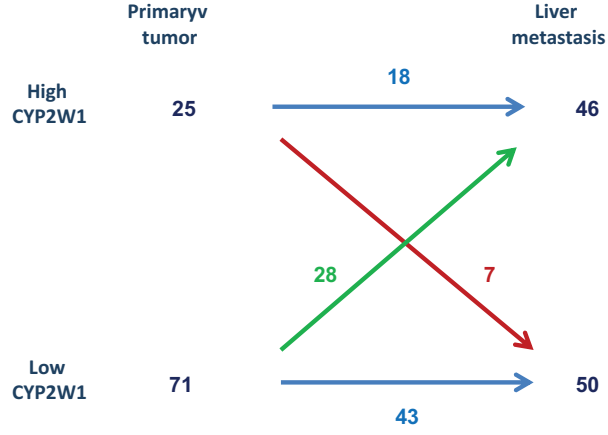

Figure 2. Change in CYP2W1 expression between primary tumor and first liver metastasis. "Low CYP2W1 expression" corresponds to the number of tumors with immunostaining grade 0–2 while "High CYP2W1" means the number of tumors with grade 3 staining. n = 96.

Discussion

This study shows that the expression of CYP2W1 increases during the progression from the primary tumor to lymph node and liver metastasis. Thus, one third of the nodal metastases and almost half of the liver metastases have high expression.

The mechanisms behind our finding are unknown, but probably related to the genotype and phenotype changes occurring in the tumor during the invasion-metastasis cascade. Studies of differences in the transcriptome between primary tumors and metastases have been performed by many groups. Habermann et al. [21] analyzed the transcriptomes and proteomes in tissue samples from 20 primary colorectal tumors and 13 liver metastases. In two cases, the primary and the metastasis came from the same patient. Total RNA from each sample was analyzed on arrays containing 9128 cDNAs. They found 158 genes being differently expressed between primary tumor and metastasis. The gene expression levels in their study correlated with chromosome copy number changes indicating that genomic instability is a major cause of the differences in gene expression. Expression level for 32 proteins increased when comparing normal tissue to metastasis [21]. However, other studies addressing the same issue have found other expression patterns with disturbing lack of overlap. Nambiar et al. [23] have suggested this to be caused by either the limited number of genes represented in the microarrays or a true biological difference. It is a problematic issue, though, to explain the expression pattern of a single gene when taking into account all the complexity of cancer genomics, transcriptomics and proteomics.

Most of the genetic aberrations seen in metastases are believed to be present already in the primary tumor. Jones and coworkers performed DNA sequencing of paired primary colorectal tumors and liver metastases from 10 patients. They found 233 mutations in the metastases, seven of which were not present in the primary tumor [22]. Ramswamy et al. report a gene expression signature of metastasis with overexpression of, e.g. genes involved in the translation apparatus, chromatin separation processes and cellular motility, already present in the primary tumor [23]. This gene expression signature was seen both in epithelial cells and stroma cells in the tumor. High expression of these genes in the primary tumor correlated with worse outcome in the patients.

We do not know the details of regulation of the *CYP2W1* gene in the primary tumor. Gomez et al. [17] have shown that methylation of a CpG island between exon one and intron one keeps the gene silenced. Changes in the methylome are a crucial event in cancer development [24]. Demethylation of this CpG island appears to be a prerequisite for CYP2W1 expression in tumors, but apparently, tissue-specific factors are also necessary since expression per se does not increase following demethylation of all types of cancer cells [17]. The events occurring during transformation of colonic epithelial cells allowing CYP2W1 expression are not known. One might assume that changes in regulatory RNA species and transcription factors also could be involved.

One possible explanation for higher prevalence of CYP2W1 expression in metastases would be up-regulation of expression in tumor cells with stem cell like properties or in cells having undergone epithelial-to-mesenchymal transition which are more likely to metastasize and thus would be enriched in a metastasis [25]. These are just speculations and not addressed in our study but are of course of interest for further examinations.

The lung metastases in our material ($n = 6$) have the same CYP2W1 expression level as both their corresponding primary tumor and liver metastases. It could reflect a true biological difference between tumors seeding metastases to the lungs compared with those that do not, or be merely a result of chance. A biological difference could be due to for instance differences in cytokine or chemokine expression in these tumors compared with those with increasing expression. Different chemokines and adhesion molecules are believed to be involved in different target organs during the metastatic process [26]. We have not addressed this issue in our study, and the number of patients with lung metastases is too small to make any conclusions.

Our study has some weaknesses. Immunohistochemistry is a semi quantitative method largely dependent on the investigators. There are also methodological problems where for instance heavy neoadjuvant treatment causes large areas of necrosis making staining interpretation more difficult. The need to validate our results using another quantitatively more accurate method, e.g. mass spectrometry, is imperative.

Furthermore, the number of patients in our study ($n = 96$) is relatively small. Our study sample is derived from a population-based cohort of 255 patients undergoing liver resection for CRC metastases in our unit during 2004–2009. Our aim was to analyze all patients, but logistically it was difficult to collect all the corresponding primary tumors that had been operated in 12 different hospitals. Thus, the current study is to be regarded as a pilot study, but has its uniqueness in the possibility to monitor the CYP2W1expression during tumor progression in the same individuals. Survival in our group of 96 patients was comparable with that of the entire

population-based cohort of 255 patients, indicating no severe skewness in our sample.

The primary tumors were resected between 1999 and 2009, a rather long time span where improvements in both diagnostic modalities, adherence to diagnostic protocols and surgical techniques have evolved. Only two of the patients were operated in 1999, and 85 (89%) of the patients had their primary resection after 2002, which increases the likelihood of the operating centers to be adherent to regional and national guidelines regarding diagnostic work-up, surgical techniques and principles for oncological treatment. In the 42 patients with a number of resected nodes smaller than 13, 21 were rectal tumors exposed to neoadjuvant treatment which could affect the number of lymph nodes in the resected specimen. Fourteen were tumors where lymph node metastases were found in spite of the lower number of nodes, and only in three cases there is a possibility of under staging because of low number of harvested lymph nodes.

The CYP2W1 enzyme is, as mentioned above, selectively expressed in transformed but not in normal cells. Analysis of the topology of the enzyme in colon cancer cells reveal that the enzyme is localized towards the luminal side of the endoplasmatic reticulum, and also consequently expressed at the outer surface of the cells [27]. About 10% of the CYP2W1 expression resides in the plasma membrane, making it also a plausible target for antibody-based treatment.

Since metastatic disease is the main cause of death in CRC, the need for complementary treatment strategies is urgent. The concept of pro drug activation described by Travica et al. [20] or an antibody-based treatment combined with our present finding that CYP2W1 is expressed in 48% of colorectal liver metastases, might indicate a new pathway that has to be explored further. Use of CYP2W1 as a drug target in the metastases might indeed be a possible way to improve future complementary treatment of CRC.

Acknowledgments

This study was possible thanks to support from The Swedish Cancer Foundation, The Ihre Foundation of the Swedish Society of Medicine, The Juhlin Foundation, Syskonen Svenssons Foundation and The Cancer Research Foundation, Radiumhemmet, Stockholm. We also thank Bengt Isaksson, Karin Ydsten and Helen Abrahamsson for good collaboration and for kindly providing data about the patients.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011; 61:69–90.

[2] Sjovall A, Jarv V, Blomqvist L, Singnomklao T, Cedermark B, Glimelius B, et al. The potential for improved outcome in patients with hepatic metastases from colon cancer: A population-based study. Eur J Surg Oncol 2004;30:834–41.

[3] Maringe C, Walters S, Rachet B, Butler J, Fields T, Finan P, et al. Stage at diagnosis and colorectal cancer survival in six high-income countries: A population-based study of patients diagnosed during 2000–2007. Acta Oncol 2013;52:919–32.

[4] National follow up colon cancer 2011. [cited 2013 Aug 19]. Available from: www.cancercentrum.se/Global/Diagnoser/kolorektal/rapporter/colon2011.pdf.

[5] National follow-up rectal cancer 2013 [cited 2013 Aug 19]. Available from: www.cancercentrum.se/Global/Diagnoser/kolorektal/rapporter/rekti2011_120629.pdf.

[6] Siegel R, DeSantis C, Virgo K, Stein K, Mariotto A, Smith T, et al. Cancer treatment and survivorship statistics, 2012. CA Cancer J Clin 2012;62:220–41.

[7] Mancini R, Ettorre G, Vennarecci G, Sperduti I, Garufi C, Esposito A, et al. Personal experience on treatment of colorectal liver metastases: A multidisciplinary approach. J Exp Clin Cancer Res 2003;22(4 Suppl):213–7.

[8] Norstein J, Silen W. Natural history of liver metastases from colorectal carcinoma. J Gastrointest Surg 1997;1:398–407.

[9] Kopetz S, Chang GJ, Overman MJ, Eng C, Sargent DJ, Larson DW, et al. Improved survival in metastatic colorectal cancer is associated with adoption of hepatic resection and improved chemotherapy. J Clin Oncol 2009;27:3677–83.

[10] Abdalla EK, Adam R, Bilchik AJ, Jaeck D, Vauthey JN, Mahvi D. Improving resectability of hepatic colorectal metastases: Expert consensus statement. Ann Surg Oncol 2006; 13:1271–80.

[11] Morris EJ, Forman D, Thomas JD, Quirke P, Taylor EF, Fairley L, et al. Surgical management and outcomes of colorectal cancer liver metastases. Br J Surg 2010;97: 1110–8.

[12] Kanas GP, Taylor A, Primrose JN, Langeberg WJ, Kelsh MA, Mowat FS, et al. Survival after liver resection in metastatic colorectal cancer: Review and meta-analysis of prognostic factors. Clin Epidemiol 2012;4:283–301.

[13] Edler D, Stenstedt K, Ohrling K, Hallstrom M, Karlgren M, Ingelman-Sundberg M, et al. The expression of the novel CYP2W1 enzyme is an independent prognostic factor in colorectal cancer – a pilot study. Eur J Cancer 2009;45: 705–12.

[14] Stenstedt K, Hallstrom M, Johansson I, Ingelman-Sundberg M, Ragnhammar P, Edler D. The expression of CYP2W1: A prognostic marker in colon cancer. Anticancer Res 2012;32: 3869–74.

[15] Karlgren M, Gomez A, Stark K, Svard J, Rodriguez-Antona C, Oliw E, et al. Tumor-specific expression of the novel cytochrome P450 enzyme, CYP2W1. Biochem Biophys Res Commun 2006;341:451–8.

[16] Wu ZL, Sohl CD, Shimada T, Guengerich FP. Recombinant enzymes overexpressed in bacteria show broad catalytic specificity of human cytochrome P450 2W1 and limited activity of human cytochrome P450 2S1. Mol Pharmacol 2006; 69:2007–14.

[17] Gomez A, Karlgren M, Edler D, Bernal ML, Mkrtchian S, Ingelman-Sundberg M. Expression of CYP2W1 in colon tumors: Regulation by gene methylation. Pharmacogenomics 2007;8:1315–25.

[18] Nishida CR, Lee M, de Montellano PR. Efficient hypoxic activation of the anticancer agent AQ4N by CYP2S1 and CYP2W1. Mol Pharmacol 2010;78:497–502.

[19] Xiao Y, Guengerich FP. Metabolomic analysis and identification of a role for the orphan human cytochrome P450 2W1 in selective oxidation of lysophospholipids. J Lipid Res 2012;53:1610–7.

[20] Travica S, Pors K, Loadman PM, Shnyder SD, Johansson I, Alandas MN, et al. Colon cancer-specific cytochrome P450 2W1 converts duocarmycin analogues into potent tumor cytotoxins. Clin Cancer Res 2013;19:2952–61.

[21] Habermann JK, Paulsen U, Roblick UJ, Upender MB, McShane LM, Korn EL, et al. Stage-specific alterations of the genome, transcriptome, and proteome during colorectal carcinogenesis. Genes Chromosomes Cancer 2007;46:10–26.

[22] Nambiar PR, Gupta RR, Misra V. An "Omics" based survey of human colon cancer. Mutation research 2010;693:3–18.

[23] Jones S, Chen WD, Parmigiani G, Diehl F, Beerenwinkel N, Antal T, et al. Comparative lesion sequencing provides insights into tumor evolution. Proc Natl Acad Sci U S A 2008;105:4283–8.

[24] Ramaswamy S, Ross KN, Lander ES, Golub TR. A molecular signature of metastasis in primary solid tumors. Nat Genet 2003;33:49–54.

[25] Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell 1990;61:759–67.

[26] Valastyan S, Weinberg RA. Tumor metastasis: Molecular insights and evolving paradigms. Cell 2011;147: 275–92.

[27] Dittmar T, Heyder C, Gloria-Maercker E, Hatzmann W, Zanker KS. Adhesion molecules and chemokines: The navigation system for circulating tumor (stem) cells to metastasize in an organ-specific manner. Clin Exp Metastasis 2008;25: 11–32.

[28] Gomez A, Nekvindova J, Travica S, Lee MY, Johansson I, Edler D, et al. Colorectal cancer-specific cytochrome P450 2W1: Intracellular localization, glycosylation, and catalytic activity. Mol Pharmacol 2010;78:1004–11.