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症例報告 II

切除不能肝転移を有する大腸癌症例に対しFOLFOX療法施行後，切除可能となった2例

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切除不能肝転移を有する大腸癌症例に対し，原発巣切除後FOLFOX4療法を施行し，肝切除し得た2症例を経験した．症例1は74歳女性．盲腸癌多発肝転移例に原発巣と肝外側区域切除後，FOLFOX4療法11コース施行し残肝腫瘍縮小効果65%にて肝右葉前区，背側区，後区域切除術施行．症例2は41歳女性．S状結腸癌多発肝転移例に5Fuを肝動注療法としたFOLFOX4療法9コース施行し，腫瘍縮小効果56%にて肝右葉切除術施行．さらにFOLFIRI3コース後に残存腫瘍摘出術施行した．両症例ともに化学療法後のPET/CTでは異常集積を認めなかったが，病理組織学的検査では残存細胞を認めた．切除不能肝転移症例に対する化学療法は延命を目的としたものが主であったが，最近では奏効率の高い全身化学療法を併用し根治切除が可能となりつつある．今後は最も適した切除時期を見極めつつ，切除後の補助化学療法も含めた集学的治療により切除不能肝転移症例の生存率の向上につながると思われる．

索引用語：切除不能肝転移，FOLFOX療法，集学的治療

大腸癌の両葉多発肝転移例の予後は不良であり，無治療例の50%生存期間は4.5～12.5カ月と報告されている[1-5]．肝切除の適応から外れた症例には姑息療法として化学療法が選択されることが多かったが，近年ではFOLFOXなどの奏効率の高い術前化学療法を施行し，down-staging後に肝切除を行い，生存率など良好な成績が報告されている[6]．今回我々は切除不能肝転移大腸癌症例に対し，原発巣切除後FOLFOX療法を施行し，肝切除し得た2症例を経験したので病理組織学的効果を含めて報告する．化学療法の腫瘍縮小効果はRECIST（Response Evaluation in Solid Tumors）ガイドライン，有害事象はNCI-CTC（National Cancer Institute Common Toxicity Criteria）に従った．組織学的腫瘍効果判定基準は大腸癌取扱い規約第7版に従った．

症 例 1

症例：74歳，女性．

主訴：右下腹部腹痛．

既往歴：特記すべき事なし．

現病歴：平成17年9月に腹痛出現し近医受診，精査にて盲腸癌，多発肝転移と診断され当科入院となった．

入院時現症：眼瞼結膜に貧血を認め，右下腹部に圧痛を認めた．

検査成績：Hb 10.9g/dlと貧血を認め，腫瘍マーカーはCEA 292ng/ml，CA19-9 307.3U/mlと高値を認めた．その他は特記すべき異常は認めず．

治療経過：入院後10月21日に肝動注ポート留置術施行．初回手術として右半結腸切除術（D3郭清），肝外側区域切除術施行．肉眼所見はC，2型，SE（A2）5.4×8.2cm，N2，H3 P0 M（－），Cur C，病理学的所見ではmod，se，int INFγ，ly0，v0，n2，CY（－），Stage IVであった．第14病日より肝動注5-Fu 500mg/日計4日施行後，左上腹部痛と肝動注ポートの閉塞を認めた．症状改善後の第22病日よりFOLFOX4療法（5Fu 1,500mg/日持続静注，5Fu 500mg/日静注，アイソボリン125mg/日点滴静注，オキザリプラチン100mg/日点滴静注）を計11コース施行した．主な有害事象はnausea Grade2，anorexia Grade 2，leukocytes Grade1であった．腫瘍マーカーは初回手術後約3カ月目に正常化し，腹部CT所見では肝転移巣の腫瘍長径和は65%の縮小率でPRとなった（Fig. 1A，B）．術前FDG-PETにて異常集積を認

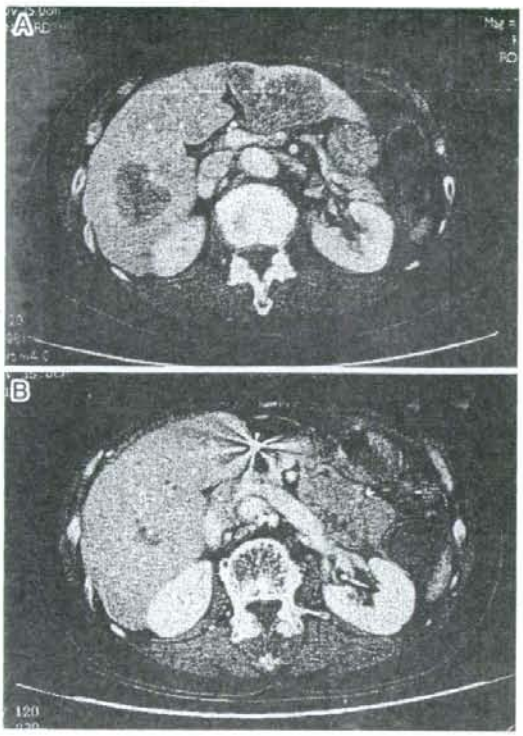

Fig. 1 CT. A. Before chemotherapy. Multiple metastatic lesions were observed in both liver lobes. B. After chemotherapy. A marked tumor reduction (65%) was observed.

めていた肝転移巣は，化学療法施行後集積像を認めなかった（Fig. 2A，B）．2006年9月12日肝右葉前区，背側区，後区域切除術施行した．

切除標本の病理組織学的所見：切除標本は275gで表面からは腫瘍は触知できず．割面標本での腫瘍部位は瘢痕組織様になっていた（Fig. 3A，B）．ルーペ像は腫瘍の繊維化を認め明らかな癌病巣は認めなかった（Fig. 4）．高倍率では変性，繊維化組織内に僅かな残存癌細胞を認め，pyknosis や karyolysis をともなう変性細胞であった．組織学的効果は Grade2 と判定した（Fig. 5）．

症 例 2

症例：41歳，女性．

主訴：肝機能障害，下腹部痛．

既往歴：38歳不安神経症．

現病歴：平成17年8月に通院中の前医にて肝機能異常，下腹部痛を指摘され精査にてS状結腸癌，多発肝転移と診断され当科入院となった．

入院時現症：眼瞼結膜に貧血を認め，左下腹部に圧痛を認めた．

検査成績：Hb 10.9g/dl，GOT 65IU/l，GPT 29IU/l，LDH 1,246IU/l．貧血と肝機能異常を認め，腫瘍マーカーは CEA 1,280ng/ml，CA19-9 390U/ml と高値を認めた．その他は特記すべき異常は認めず．

治療経過：入院後9月15日に初回手術としてS状結腸切除術（D3郭清），IVH ポート留置術施行．肉眼所見は S，2型，SE（A2）3.3×2.0cm N2，H3，P1，Cur C，病理学的所見では well＞mod，se，int，INFγ，ly1，v1，n2，CY（＋）Stage IV であった．9月22日肝動注ポート留置術施行．第8病日より 5Fu のみ肝動注を用いた FOLFOX4 療法（5Fu 1,800mg/日持続動注 5Fu 500mg/日動注，アイソボリン 150mg/日点滴静注，オキザリプラチン 120mg/日点滴静注）を計9コース施行した．主な有害事象は nausea Grade1，anorexia Grade3 であった．腫瘍マーカーは初回手術後約4カ月目に正常化し，腹部 CT 所見では肝転移巣の腫瘍長径和は56％の縮小率で PR となった（Fig. 6A，B）．FDG-PET では異常集積認めず，有害事象も強くなってきたため肝転移巣に対し，2期的手術を予定とし2006年4月27日肝右葉切除術施行した．全身状態改善後の6月13日より FOLFIRI 療法（5Fu 3,360mg 持続静注，5Fu 560mg/日静注，アイソボリン 280mg/日点滴静注，CPT-11 210mg/日静注）計3コース施行した．主な有害事象は diarrhea Grade1，nausea Grade1 であった．腹部 CT 所見では肝転移巣の腫瘍長径和は20％の縮小率で SD であり（Fig. 6C），9月8日肝 S3 亜区域切除，S2 腫瘤摘出術施行した．

切除標本の病理組織学的所見：初回手術の切除標本は556gで表面からは腫瘍は触知できず．割面標本での腫瘍部位は瘢痕組織，壊死様になっていた（Fig. 7）．粘液様に変性した組織内に癌病巣を認めたが，残存癌細胞は pyknosis や karyolysis をともなう変性細胞を多く認めた．組織学的効果は Grade2 であった．2回目の手術は切除標本は118gであり，化学療法による変性細胞は1/3以下であり，多数の残存癌細胞を認めた．組織学的効果は Gradela であった．

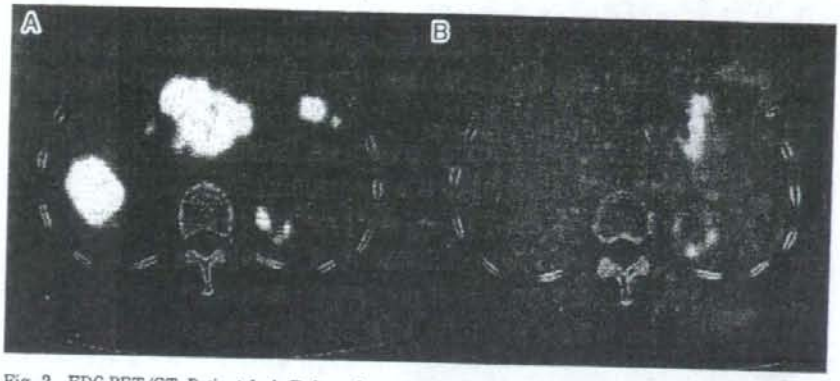

Fig. 2 FDG-PET/CT. Patient 1. A. Before chemotherapy. Abnormal uptake was seen in multiple metastatic lesions in both liver lobes. B. After chemotherapy. No abnormal uptake was observed.

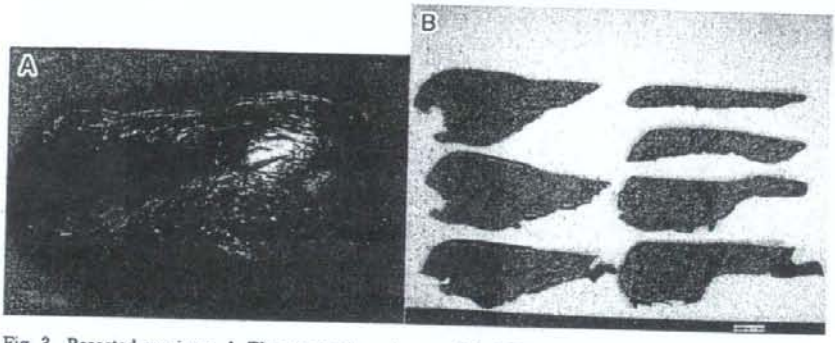

Fig. 3 Resected specimen. A. The resected specimen weighed 275g. No tumors were palpable from the surface. B. On the cut surface, the tumors had turned into scar tissue.

Fig. 4 Histopathological findings. A. Loupe image. Tumor fibrosis and mucousnization were observed, but there was no evidence of cancer lesions.

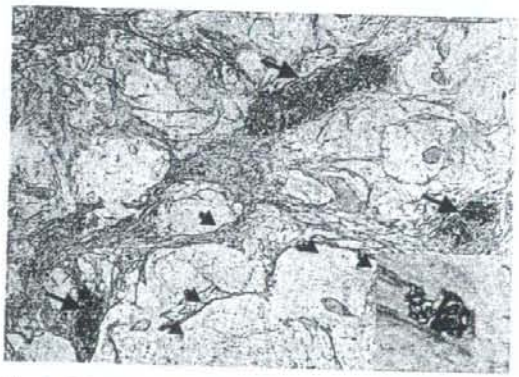

Fig. 5 Only a few tumor cell clusters (arrowheads) are present in mucous pools of metastases. Foci of calcification (arrow) are scattered (HE×40). Most of the residual tumor cells are markedly degenerative with pyknosis or Karyolysis, although some of these are regarded viable (HE×400).

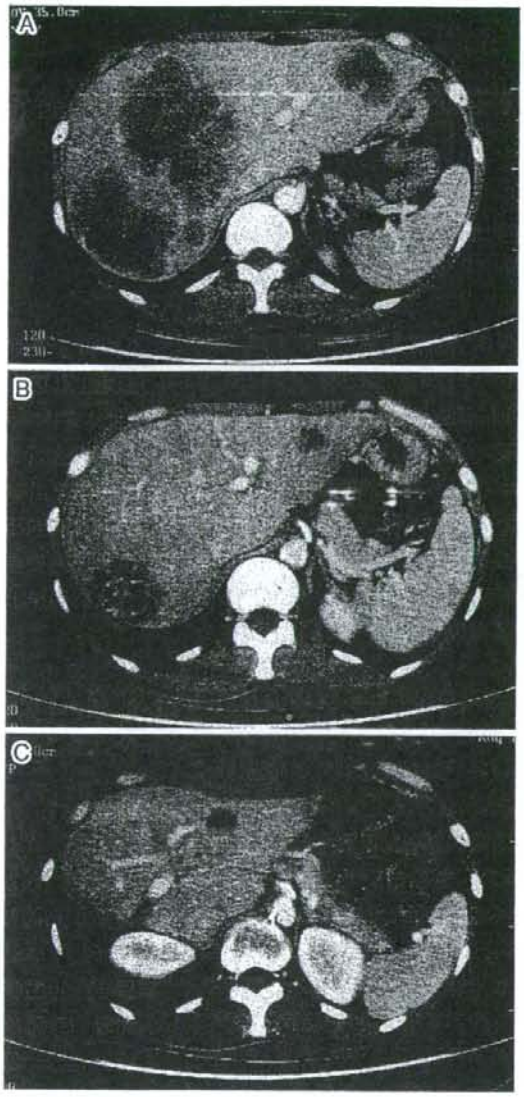

Fig. 6　CT. A. Before chemotherapy. Multiple metastatic lesions were observed in both liver lobes. B. After FOLFOX therapy. A marked tumor reduction (56%) was observed. C. After FOLFIRI therapy. A 20% tumor reduction was noted.

考　察

大腸癌のうち肝転移は同時性に10%，異時性に15%と最も高頻度に認める転移形式である[7]．無治療例の50%生存期間は4.5～12.5カ月と報告されている[1-5]．肝転移例に対する治療法は切除療法が良好であると報告されているが[8]，Ballantyneらは単発あるいは少数個（3個）転移までが積極的切除の適応と述べている．これらの適応に含まれる症例は大腸癌全体の5%であり，肝切除による生存率の向上は1～2%と述べられている[9]．そのため生存率を改善させるためには適応外の症例の生存率を改善させる事が全体の生存率が向上すると思われる．海外では切除不能，あるいは肝外病変をともなう大腸癌遠隔転移例に対し，積極的に奏効率の高いFOLFOXをneoadjuvant chemotherapyとして用い，切除率を向上させている[6,10]．大腸癌肝転移切除不能例に対する術前化学療法の意義は転移病巣の縮小により手術が可能になる事であり，術前化学療法の奏効率と切除率は相関すると述べられている[11]．またAdamsらは，術前化学療法後に切除可能となった症例の5年生存率は，診断時に切除可能であった症例の切除成績と同等であると述べている[12]．今回我々の経験した2症例は術前に切除不能の多発肝転移例であり，腹痛を認め，肝転移が生命予後を規定すると考えられたため，原発巣切除後にFOLFOX4療法を施行した．

投与方法として肝動注療法は肝転移例の治療法の選択肢の1つとして用いられているが，フッ化ピリミジン系薬剤を用いた全身化学療法と肝動注化学療法との無作為比較試験では肝動注療法での奏効率は高いものの生存期間中央値に有意差は認めなかった[13,14]．Araiらは肝動注療法の奏効率は60～80%で，奏効例の50%生存期間は約2年と報告している[15]．肝臓に対する治療効果は高いものの肝外病変のコントロールができないのが問題であるとされている．当科における肝動注療法の基本的な考えは，本症例のように，高度な肝転移巣や，それ以上に肝臓に転移巣が占めているような症例に対して，原発巣が腹痛や，腸閉塞症状，出血などによる貧血を認める場合は原発巣に対する切除術を施行し，術後肝機能障害などから肝不全などに陥る可能性のある症例に対し5Fuのみ肝動注療法を併用したFOLFOX療法を施行し，治療効果や有害事象などにより全身療法へ移行している．今回我々の経験した2症例は肝転移が非常に高度であり，原発巣切除術後に肝機能障害から肝不全に陥る可能性も考えられ，肝転移が生命予後を規定すると考えられたため，肝臓への治療効果の高い肝動注療法を併用したFOLFOX4療法を施行した．1例目は肝動注ポートの閉塞によ

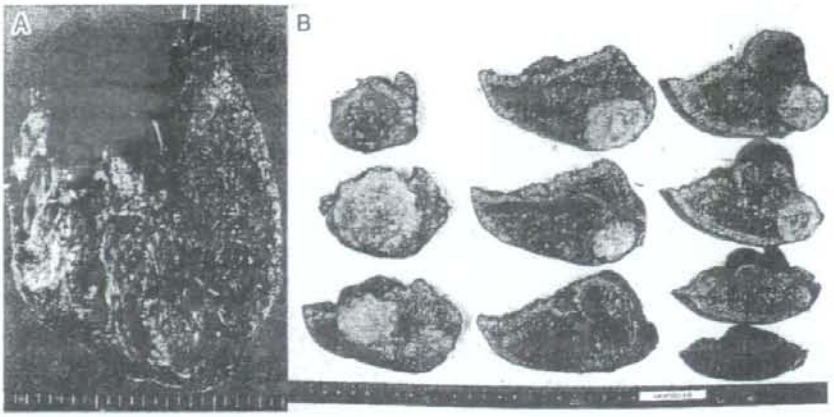

Fig. 7　Resected specimen. A. The initially resected specimen weighed 556 g, and no tumors were palpable from the surface. B. On the cut surface, the tumors had turned into scar tissue and become necrotic.

り全身化学療法へと移行せざるを得なかったが，2例目は計9コース施行可能であった．

2症例ともに投与方法は異なるが3～4カ月後には腫瘍マーカーも正常化し，CTにても各々約60%の腫瘍縮小効果を認め切除術を施行した．

切除標本の組織効果は2例ともに変性，繊維化組織内に残存癌細胞を認め，pyknosisやkaryolysisをともなう変性細胞であり，Grade2と判定した．1例目はFDG-PETにて集積を認め無かったが，僅かではあるが癌細胞の残存を認めていた．2例目も2回目の切除病変はFDG-PETでは集積を認めていなかったが，切除標本上は癌細胞の残存を認めていた．

Marcusらは同時性肝転移症例14例34病変にneoadjuvant chemotherapyを施行し，FDG-PETにて集積像を認めなくなった病変のうち3例(21%)，5病変(15%)のみ病理学的にCRであったと述べている[16]．大腸癌におけるFDG-PET診断能は原発巣でのsensitivityは95～100%と高率であるが，specificityは43%と高くはない[17,18]．局所再発，肝転移のsensitivity，specificity，accuracyは90%以上と報告されている[19]．診断精度は組織密度による影響が強く，Markらは粘液癌でのsensitivityは約60%と低値であると報告し，粘液成分の多い粘液癌では細胞密度が相対的に低くなりsensitivityが低下すると述べている[20]．我々の2症例も化学療法施行後は残存病変を認めたが，繊維化，粘液様に変性しており集積像を呈さない可能性も考えられた．また，Beoistらは肝転移症例に対し化学療法施行しCT画像上CRとなった46病変のうち15病変を切除したが12例(80%)に遺残癌細胞を認め，非切除31病変のうち23病変(74%)に再発を認めたため，画像上CRを認めても可能であれば切除療法をすべきであり，他の治療法としても局所再発などを減らすべく全身化学療法や，肝動注療法など積極的な治療法を選択するべきと述べている[21]．

また，切除標本においてオキザリプラチンによる肝臓の毛細血管の変性や脂肪蓄積について報告され[22]，AdamらFOLFOX療法施行後切除した肝組織の病理所見はLV/5Fu症例よりも繊維化や脂肪蓄積所見を多く認めたと報告している[23]が，2症例では繊維化を認めるのみであった．

初回手術後約2年が経過しているが1例目は690日目に肺再発を，2例目は623日目に残肝再発を認め，1例目はFOLFOX4療法を，2例目はFOLFIRI療法を再度施行中である．Stevenらは切除不能肝転移症例にFOLFOX療法を施行し，切除し得た症例の約70%に再発を認め，特に残肝再発が多く認めたと報告している[8]．残肝を含めた切除後の補助化学療法は今後の治療方針として重要と思われる．

以前までは切除不能肝転移症例に対し延命を目的とした化学療法が主であったが，最近では我々が経験した2症例のように奏効率の高い全身化学療法を併用することで根治切除が可能となりつつある．今後は肝切除し得た症例の術後の補助化学療法も含め，最も適した切除時期を見極めつつ，集学的治療を施行することで切除不能肝転移症例の生存率の向

上を図ることが重要と思われる．

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●原 著●

高度な肝機能障害を伴い切除不能多発肝転移を有する大腸癌症例に対する肝動注併用 FOLFOX 療法の検討

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Combination of Hepatic Arterial Infusion Therapy and FOLFOX for Colorectal Cancer with Multiple Unresectable Liver Metastases Causing Severe Liver Dysfunction: Takeshi Suto[*1], Toshihiko Sato[*1], Naoki Mori[*1], Naruhisa Takano[*1], Koshiro Ishiyama[*1], Naoki Sakurai[*1], Kiyohiro Saito[*2], Hajime Iizawa[*1] and Eiichi Ikeda[*1] (*Dept. of [*1]Gastroenterological Surgery, [*2]Radiology, Yamagata Prefectural Central Hospital*)

Summary

Purpose: The purpose of this study was to evaluate the efficacy of the combination of hepatic arterial infusion therapy and FOLFOX for colorectal cancer with multiple unresectable liver metastases causing severe liver dysfunction.

Subjects and Methods: The subjects were 13 colorectal cancer patients who had undergone resection of the primary tumor, and showed multiple, unresectable liver metastases and severe liver dysfunction. They consisted of 8 men and 5 women, with a median age of 63 (29-77) years. Of these patients, 7 and 6 had colon and rectum cancers, respectively. They had an average of 8 (3-22) liver metastases of 4.6 (1.5-14.5) cm in diameter. During surgery, extrahepatic lesions were found in 3 patients (P in 2, and CY in 1). The preoperative serum LDH and ALP levels were high, at 1,099 (322-1,418) and 1,011 (644-2,384), respectively. The follow-up period was approximately 500 (248-928) days. Only 5-FU in FOLFOX4 or 6 m therapy was infused into the hepatic artery, and LV and L-OHP were injected into the central venous port about every two weeks. Response rates and adverse events were evaluated according to the RECIST criteria and CTCAE ver 3.0, respectively.

Results: The therapy was performed 14 (6-22) times, with a response rate of 84.6% for liver metastases, facilitating hepatectomy in 1 patient. The overall response rate was 61.5%, with 1 patient dying of the primary cancer on the 265th day. Grade 3 adverse events were neutropenia and anorexia in only 1 patient each, and no adverse events were specific to hepatic arterial infusion.

Conclusion: Since the follow-up period after this therapy was still short, only 13 patients have received the therapy. However, it appears that it can be performed relatively safely, and is effective for the control of extrahepatic lesions as well. Therefore, this therapy provides good control, and can be a treatment option. Key words: Colorectal cancer, Multiple liver metastases, Hepatic arterial infusion, FOLFOX (*Received Apr. 2, 2008/Accepted Jul. 3, 2008*)

要旨 目的: 高度な肝機能障害を伴う切除不能多発肝転移を有する大腸癌症例に対する肝動注併用 FOLFOX 療法の有効性について検討する。対象と方法: 高度な肝機能障害を伴う切除不能多発肝転移を有し，原発巣を切除した大腸癌症例13例を対象とした。男性8例，女性5例，年齢は中央値63（29～77）歳であった。結腸7例，直腸6例，肝転移個数は8（3～22）個，大きさ4.6（1.5～14.5）cmであり，術中肝外病変は3例（P 2例，CY 1例）に認めた。術前血中 LDH 1,099（322～1,418），ALP 1,011（644～2,384）と高値であった。観察期間は約500（248～928）日であった。FOLFOX4 または6m療法の5-FUのみ肝動注より動注し，LV と L-OHP は中心静脈ポートより静注し，約2週間ごとに施行した。奏効率は RECIST に，有害事象は CTCAE ver 3.0 に従い評価した。結果: 施行回数は14（6～22）回であった。肝に対する奏効率は84.6%で，1例に切除可能であった。全体では61.5%の奏効率であり，死亡例は1例（265日目原癌死）であった。grade 3の有害事象は neutropenia 1例，anorexia 1例のみで肝動注特有の有害事象は認めなかった。まとめ: 肝動注併用 FOLFOX 療法は観察期間がまだ短く，症例数が13例と少ないものの比較的安全に施行でき，肝外病変のコントロールも含めて比較的有効と思われるため，局所制御の良好な肝動注療法を併用した FOLFOX 療法は治療法の選択肢になり得ると思われた。

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はじめに

大腸癌のうち肝転移は同時性に10%，異時性に15%と最も高頻度に認める転移形式である[1]。大腸癌の両葉多発肝転移例の予後は不良といわれ，無治療例の50%生存期間は4.5～12.5か月と報告されている[2-6]。かつて本邦においてはAraiらにより5-fluorouracil（5-FU）単剤による肝動注療法が施行され，良好な局所制御効果を認めていたが[7]，欧米での全身化学療法とのランダム化比較試験においては生存期間延長効果を認めないと報告されていた[8]。近年ではFOLFOXなどの奏効率の高い全身化学療法を施行し，down-staging後に肝切除を行い，生存率など良好な成績が報告されている[9]。そのため今回われわれは，高度の肝機能障害を有する切除不能な大腸癌肝転移症例に対し，局所制御効果の高い5-FUの肝動注療法にlevofolinate calcium（LV）とoxaliplatin（L-OHP）の全身投与との併用療法を施行し，その有効性と安全性について検討した。化学療法の腫瘍縮小効果はRECIST（Response Evaluation in Solid Tumors）ガイドライン，有害事象はCTCAE ver. 3.0（Common Terminology Criteria for Adverse Events v3.0）に従った。組織学的腫瘍効果判定基準は大腸癌取扱い規約第7版に従った。

Ⅰ．対象および方法

1．対　象

2005年6月～2007年8月までに切除不能肝転移を有する大腸癌症例に対し，当科にて原発巣切除後first-lineにてFOLFOX療法を施行した進行大腸癌症例65例中，高度の肝機能障害を有する多発肝転移にて術後，全身状態の悪化が考えられた13例を対象とした。13例の臨床病理学的特徴をTable 1に示す。肝外病変を有する症例は3例（術中所見にて腹膜播種2例，肝門部リンパ節腫脹1例）に認めた。術前ALP値の中央値は1,011（644～2,384）と高値であった。

Table 1　Subjects

Gender	
Male/female	8/5
Age	63（29～77）
Performance status	
0/1/2/3/4	11/2/0/0
Tumor location	
Colon/rectum	7/6
Number of liver metastases	8（3～22）
Diameter of liver metastases（cm）	4.6（1.5～14.5）
Extra hepatic metastases	
yes/no	3/10
P/CY	2/1
GOT（IU/L）	104（31～228）
GPT（IU/L）	110（32～208）
γ-GTP（IU/L）	252（85～631）
T-Bill（mg/mL）	0.8（0.3～1.3）
LDH（IU/L）	1,099（322～1,418）
ALP（IU/L）	1,011（644～2,384）
CEA（ng/mL）	362.1（65.6～3,832）
CA19-9（U/mL）	451.3（46.9～73,816）

2．方　法

術前に放射線科医師によりIVRにて胃十二指腸動脈と右胃動脈の血流改変術を施行し，同時期に右大腿動脈から肝動脈内にカテーテルを留置し，ポートを皮下に埋め込んだ。術中に外科医師により鎖骨下静脈より中心静脈ポートの留置を施行した。投与方法はFOLFOX4または6 mのレジメンと同様に施行したが，5-FUのみ肝動注ポートより注入し，LVとL-OHPを中心静脈ポートより注入した。約2週間ごとにPDまたは全身状態悪化，有害事象にて中止となるまで施行した。

Ⅱ．結　果（Table 2，3）

1．投与状況

肝動注併用FOLFOX療法の施行回数は中央値で14（6～22）回あった。4例が治療継続中であり，中止の理由は1例が肝臓切除により，3例がPDにより，1例が肝動脈閉塞により，4例が有害事象などであった。

Table 2　Response rate and prognosis

Response	Liver	Overall
Complete response, No	0	0
Partial responses, No	11	9
Stable diseanse, No	1	1
Progressive diseases, No	1	4
Response		
No.	11	8
%	84.6	61.5
Death		
No（days after chemotherapy）		1（265 days）
Courses of chemotherapy		14（6～22）

Table 3　Adverse events

Adverse events	grade			2～3 No.（%）
	1	2	3	
Neutropenia	1	1	1	2（15.3）
Hb	1	0	0	0（0）
Platelet	1	0	0	0（0）
Anorexia	1	0	1	1（7.7）
Nausea	1	1	0	1（7.7）
Diarrhea	1	0	0	0（0）
Paresthesias	7	2	0	2（15.3）
Allergy	0	0	0	0（0）

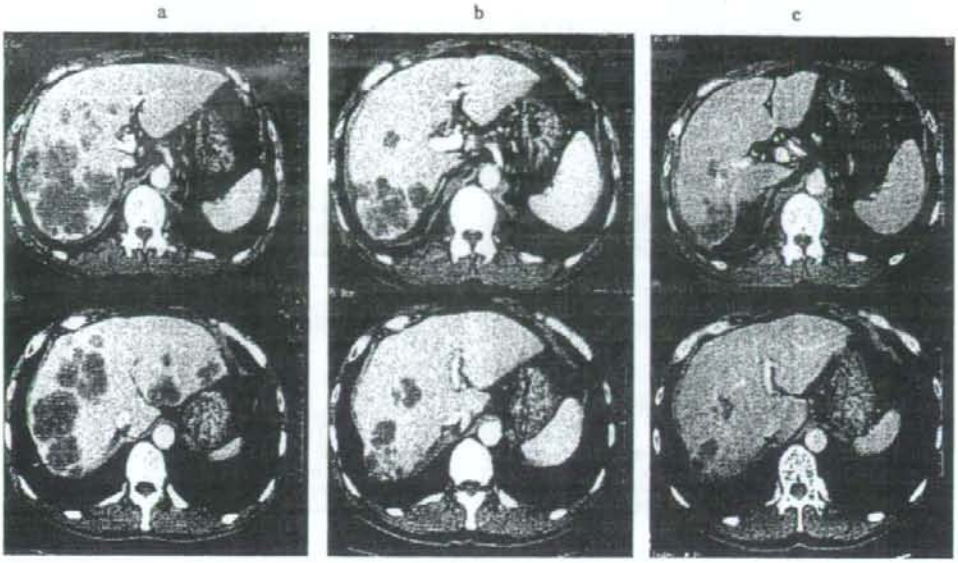

Fig. 1 Case 1
a: Before surgery and chemotherapy.
b: After 6 courses of combined hepatic arterial infusion therapy and FOLFOX6m.
c: After 16 courses of therapy. Reduction rate: 61%.

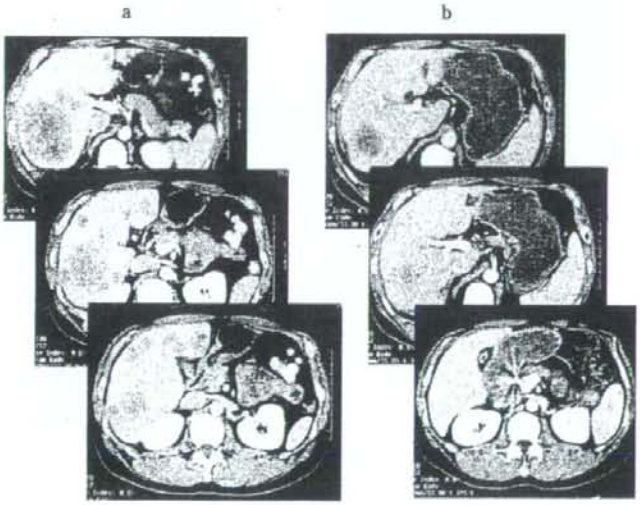

Fig. 2 Case 2
a: Before surgery and chemotherapy.
b: After 17 courses of combined hepatic arterial infusion therapy and FOLFOX6m. Reduction rate: 72%.

2．抗腫瘍効果

肝臓病変における抗腫瘍効果はCRを認めないものの約85%と高率であり，Fig. 1～4に著効例を示すが，Fig. 4は著効後に切除可能例となり，Fig. 2は今後切除予定である。臨床的奏効率は61.5%であり，肝病巣の悪化と他病巣の出現により4例にPDを認めたが比較的肝外病変のコントロールも良好であった。

3．予後と後治療について

死亡例は1例のみで投与後265日であった。投与後観察期間が中央値で495（248～928）日と短いこともあるが，2年以上生存例は2例，1年以上生存例は5例と予後は比較的良好であった。2次治療として8例にFOLFIRI療法を，切除可能例は切除後S-1内服を施行している。

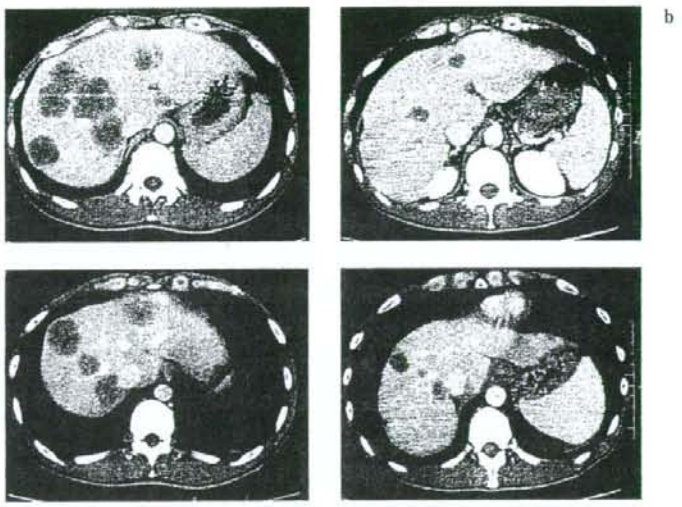

Fig. 3 Case 3
a: Before surgery and chemotherapy.
b: After 9 courses of combined hepatic arterial infusion therapy and FOLFOX6m. Reduction rate: 68%.

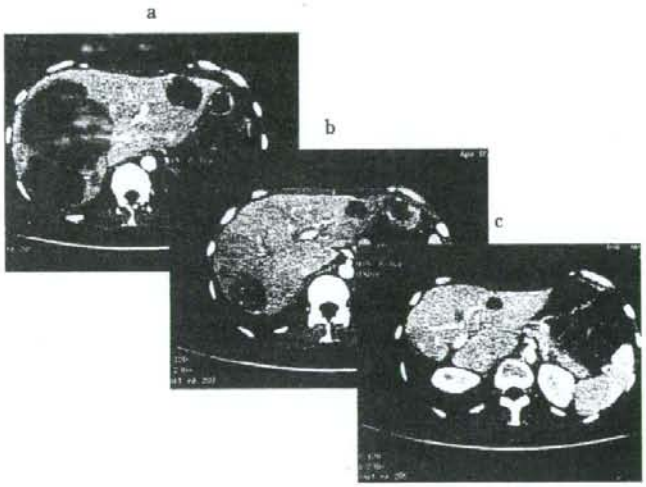

Fig. 4 Case 4
a: Before chemotherapy. Multiple metastatic lesions were observed in both liver lobes.
b: After 9 courses of combined hepatic arterial infusion therapy and FOLFOX4. A marked tumor reduction (56%) was observed and tumor was resected.
c: After 3 courses of FOLFIRI therapy. A 20% tumor reduction was noted and rest tumor was resected.

4. 有害事象

grade 3 以上の有害事象例は好中球減少例 1 例と anorexia 1 例であり，grade 2 は重複を含むものの nausea など 2 例と L-OHP に特有の末梢神経障害 2 例であった。重篤なアレルギーなどは認めなかった。

Ⅲ. 考　察

肝転移例に対する治療法は切除療法が良好であると報告されているが[10]，Ballantyne らは単発あるいは少数個（3 個）転移までが積極的切除の適応と述べている。これらの適応に含まれる症例は大腸癌全体の 5%であり，肝切除による生存率の向上は 1～2%と述べられている[11]。

そのため，生存率を改善させるためには適応外の症例の生存率を改善することが全体の生存率を向上させると思われる。

海外では切除不能，あるいは肝外病変を伴う大腸癌遠隔転移例に対し，積極的に奏効率の高いFOLFOXをneoadjuvant chemotherapyとして用い，切除率を向上させている[9,12]。大腸癌肝転移切除不能例に対する術前化学療法の意義は転移病巣の縮小により手術が可能になることであり，術前化学療法の奏効率と切除率は相関すると述べられている[13]。またAdamsらは，術前化学療法後に切除可能となった症例の5年生存率は，診断時に切除可能であった症例の切除成績と同等であると述べている[14]。

本邦では1990年代前半には確立されていた肝動注療法は全身化学療法との比較試験において，腫瘍縮小効果において勝るものの生存期間の延長において優位性が示されず，肝外病変の増悪の抑制が弱く，カテーテル留置の手技的困難性よりfirst-lineとして活用されなくなっていた。しかし，これらの検討では本邦において施行されていた肝動注療法と異なりカテーテル留置が開腹下で施行されており，肝動注群の37%で治療開始できず，治療開始例の29%でカテーテルトラブルにて治療継続不可能であり，最終的には6コース予定に対し，平均2コースの治療しか行われていなかった[8]。

本邦においては5-FUを週1回5時間かけて注入する治療法で奏効率は約50～80%，生存期間中央値は18～26か月と良好な成績であった[7,15,16]。ランダム化比較試験は行われていないものの，近年のFOLFOXやFOLFIRIといった全身化学療法と差を認めていなかった。さらに最近の肝動注療法の報告では，山下らはweekly 5-FU+LV肝動注療法は肝病変に対する奏効率は75%で生存期間中央値は22か月と報告し[3]，KemenyらはFUDRの肝動注療法と5-FU+LV全身療法の比較試験において奏効率が47%と24%，生存期間中央値が24.4か月と20.0か月で有意に肝動注療法が勝っていると報告している[17]。

これらよりALPの高値など高度な肝機能障害を伴う切除不能肝転移を有する大腸癌に対する化学療法として，本邦の放射線科医師による高い技術のもとカテーテル留置を施行し，肝転移に対する腫瘍縮小効果の高い肝動注療法と，肝外病変の制御のため全身療法を併用することで予後の改善が得られると考えられるため，今回われわれは切除不能な大腸癌高度肝転移症例に対し5-FUのみ肝動注ポートより注入し，LVとL-OHPを中心静脈ポートより注入する治療法を13例に施行した。L-OHPが本邦において承認されてから期間がまだ短いため，観察期間の中央値が約500日と短いものの，肝病変に対する奏効率は約85%と高率であり，1例に切除可能で，さらに1例に切除予定であった。肝外病変も含めても約62%の奏効率と良好であり，比較的肝外病変のコントロールもされていると考えられた。また，13例全例に留置可能で1例のみにカテーテル閉塞を認めたのみであった。肝動注併用FOLFOX療法の施行回数は中央値で14（6～22）回であったが，grade 3以上の有害事象は好中球減少症とanorexiaの2例のみで，grade 2は重複を含むものの4例であり，肝動注療法に特異的な胆嚢炎や胃十二指腸潰瘍などは認めず，比較的安全に施行されていた。L-OHPに特異的な重度の末梢神経障害や，アレルギーも認めていなかった。予後においては，観察期間が短いものの後治療としてFOLFIRIやS-1の内服が施行されているが，死亡例は265日目の1例を認めたのみであった。

欧米において，Ducreuxらは薬剤分布が適当であっても腹痛を引き起こしたものの，L-OHPを肝動注に用い，5-FU+LVを全身化学療法とし，奏効率64%，MST約27か月と報告し[18]，KemenyらはFUDRの肝動注と，irinotecan，L-OHPの全身療法により奏効率90%，MST約36か月と報告している[19]。成績の向上は後治療の分子標的治療薬なども考慮しなければならないものの，肝動注化学療法と全身化学療法とを併用することで，現在最も施行されている標準的全身化学療法のFOLFOX，FOLFIRI療法のMST約20か月よりも優れた成績を示す可能性が考えられるため，今後はこれらを対照としたランダム化試験も必要と思われる。

今回われわれは，肝機能障害を有する切除不能多発肝転移症例に対し，肝転移に関する局所治療としての肝動注療法と，肝外病変のコントロールとして全身化学療法を併用としたFOLFOX療法を13例に施行した。観察期間が短く今後の長期的観察が必要であるが，奏効率や肝外病変に対するコントロールは比較的良好であり，安全に施行されていた。以前までは切除不能肝転移症例に対し延命を目的とした化学療法が主であったが，最近ではわれわれが経験した症例のように高度な肝機能障害を有する症例に対しても奏効率の高い肝動注療法と全身化学療法を併用することで根治切除が可能となり，治療法の一つの選択肢となる可能性が示唆された。今後は肝切除し得た症例の術後の補助化学療法も含め，集学的治療を施行することで切除不能肝転移症例の生存率の向上を図ることが重要と思われる。

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5-Fluorouracil-related Gene Expression in Hepatic Artery Infusion-treated Patients with Hepatic Metastases from Colorectal Carcinomas

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Abstract. *Aim: To predict the therapeutic efficacy of hepatic arterial infusion (HAI) with 5-fluorouracil (5FU) for patients with liver metastases from colorectal carcinomas, 5FU-related gene expressions were examined in primary colorectal carcinomas. Patients and Methods: Thirty-eight patients with liver metastases from colorectal carcinoma received HAI of 5FU. The expressions of the mRNAs for thymidine synthase (TS), dihydropyrimidine dehydrogenase (DPD), thymidine phosphorylase (TP), and oroteta phophoribosyl transferase (OPRT) in primary colorectal carcinomas were measured by RT-PCR. Results: The response rate was 52.6% (20/38). The overall median survival time was 29.1 months. DPD and TP expression was significantly higher in the progressive disease (PD) group than in the complete response (CR) or partial response (PR) group ($p=0.032$, $p=0.014$), respectively. The levels of DPD and TP mRNAs showed a significant correlation ($r=0.76$, $p=0.0001$). Conclusion: The expression of DPD and TP mRNAs in primary colorectal carcinomas was significantly predictive of the therapeutic response to 5FU HAI.*

Hepatic metastasis is one of the most important factors that determines the prognosis of patients with advanced colorectal carcinoma. Surgical resection alone can result in significant prolongation of survival in patients with favorable prognostic factors (1, 2). Systemic chemotherapy regimens that include 5-fluorouracil (5FU) have been used to treat hepatic metastases in colorectal carcinoma patients when surgical resection cannot be performed (3, 4).

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Key Words: Thymidine synthase, dihydropyrimidine dehydrogenase, thymidine phosphorylase, oroteta phophoribosyl transferase.

Hepatic artery infusions (HAIs) have also been performed as regional chemotherapy for liver metastases arising from colorectal carcinomas. Randomized trials evaluating HAI therapy for the treatment of unresectable hepatic metastases have demonstrated higher response rates (31%-50%) than those achieved with systemic chemotherapy (8%-20%), but no survival benefit was reported (5, 6). Recently, Kemeny and associates have reported the results of a randomized trial comparison between HAI using floxuridine and systemic chemotherapy using 5FU and leucovorin (7). The overall survival was significantly longer for HAI than the systemic treatment (median, 24.4 *vs.* 20 months).

In a previous study, we administered 5FU by HAI to patients with liver metastases from colorectal carcinoma after radiological placement of the infusion lines, and found that HAI significantly improved the median survival time (MST) and response rate (8). We also reported that lymph node metastases in primary carcinoma and the pre-treatment serum CEA level were prognostic factors for MST in HAI-treated patients.

However, the response rate was not influenced by the histological features or lymph node metastases of the primary colorectal carcinomas, nor was it influenced by the synchronous/metachronous status of the liver metastases, the number of hepatic metastases, or the pre-treatment serum CEA levels.

It has been reported that enzymes involved in 5FU metabolism, such as thymidine synthase (TS), dihydropyrimidine dehydrogenase (DPD), thymidine phosphorylase (TP) and oroteta phophoribosyl transferase (OPRT) are important predictors of the therapeutic efficacy of 5FU (9, 10). TP, also known as platelet-derived endothelial cell growth factor, plays an important role in the angiogenesis of carcinomas. It has been reported that the clinical response and survival rates in response to 5FU-based chemotherapy for colorectal carcinomas are related

to the expressions of TS, DPD and TP and that a high level of TP gene expression in colorectal carcinomas is associated with non-responsiveness to 5FU (9, 11, 12). The expression of these enzymes is important for guiding the rational selection of chemotherapeutic regimens. The expression of TS, DPD, TP, and OPRT genes has been examined by a newly developed technique using laser-captured microdissection combined with RNA extraction from paraffin-embedded specimens (13-16).

The expression of enzymes involved in 5FU metabolism has not been examined in patients with liver metastases treated using HAI. The aim of this study was to investigate the correlation between the clinical response to HAI and the expression of TS, DPD, TP and OPRT mRNAs in primary colorectal carcinomas.

Patients and Methods

Patients. Patients with liver metastases originating from colorectal carcinomas were included (n=38). Patients characteristics are described in Table I. Their primary colorectal carcinomas had been resected surgically and were histologically confirmed. Patients with extrahepatic metastases were excluded. The patients received no other chemotherapy prior to HAI. Informed consent was obtained from all patients.

Catheter placement and HAI procedure. Catheter placements in the hepatic artery were performed radiologically by interventional radiologists using the distal fixation method (17). The catheter was inserted *via* the right femoral artery and connected to the infusion port (Infuse-a-Port, Strato Medical Corp., Beverly, MA, USA). The HAI treatment was performed weekly or every 2 weeks at an outpatient chemotherapy facility. The 5FU (1,000-1,500 mg) was dissolved in 200 ml of physiological saline and loaded into a portable infusion pump (Intermate LV; Baxter Healthcare Corp., Deerfield, IL, USA). HAI was performed continuously for 5 h at an infusion rate of 50 ml/h (8).

Clinical response and survival evaluation. The patients scheduled for HAI received a chest and abdominal computed tomography (CT) scan before the start of treatment. Tumor status was assessed by chest and abdominal CT scans after every 10 infusions. The therapeutic response was evaluated according to the Response Evaluation Criteria In Solid Tumors (RECIST) guideline (18) as complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD). Actuarial survival curves were computed by the Kaplan-Meier method, using GraphPad Prism version 4.0 for Macintosh (San Diego, CA, USA).

Microdissection. Four 10 μm-thick sections of the primary colorectal carcinomas and adjacent normal mucosa were prepared from the paraffin-embedded blocks. One 4 μm-thick section was prepared and stained with hematoxylin and eosin (HE). A representative formalin-fixed, paraffin-embedded (FFPE) tumor specimen was selected by a pathologist after examination of the HE-stained slides. Sections 10 μm in thickness were stained with neutral fast red to enable visualization of histology for laser capture microdissection (PALM Microlaser Technologies AG, Munich, Germany), which was performed to ensure that only tumor cells were studied.

Table I. *Patients characteristics.*

Characteristics	No. of patients	Characteristics	No. of patients
		pTNM of primary colorectal carcinoma	
Gender			
Male	25	pT	
Female	13	pT1	0
Age (average)	65.6	pT2	0
Onset of liver metastases		pT3	35
Synchronous	25	pT4	3
Metachronous	13		
Histology of primary colorectal carcinoma		pN	
		pN0	11
Well	11	pN1	15
Moderate	25	pN2	12
Poor	1	pM	
Mucinous	1	pM0	13
		pM1	25

RNA extraction and cDNA synthesis. The RNA was isolated from the FFPE specimens using a novel, proprietary procedure (Response Genetics, Los Angeles, CA, USA) (9). The tissue samples to be extracted were placed in a 0.5 mL thin-walled tube containing 400 Bl of 4 M dithiothreitol (DTT)- GITC/sarc (4 M guanidinium isothiocyanate, 50 mM Tris-HCl, pH 7.5, 25 mM EDTA) (Invitrogen; No. 15577-018). The samples were homogenized and an additional 60 Bl of GITC/sarc solution was added. They were heated at 92°C for 30 min and then transferred to a 2 mL centrifuge tube. Fifty microliters of 2 M sodium acetate was added at pH 4.0, followed by 600 Bl of freshly prepared phenol/chloroform/isoamyl alcohol (250:50:1). The tubes were vortexed for 15 sec, placed on ice for 15 min and then centrifuged at 13,000 rpm for 8 min in a chilled (8°C) centrifuge. The upper aqueous phase was carefully removed and placed in a 1.5-mL centrifuge tube. Glycogen (10 Bl) and 300-400 Bl of isopropanol were added and the samples were vortexed for 10-15 sec. The tubes were chilled at -20°C for 30-45 min to precipitate the RNA. The samples were then centrifuged at 13,000 rpm for 7 min in an 8°C centrifuge. The supernatant was poured off and 500 Bl of 75% ethanol was added. The tubes were again centrifuged at 13,000 rpm for 6 min in a chilled (8°C) centrifuge. The supernatant was then carefully poured off, so as not to disturb the RNA pellet, and the samples were quick-spun for another 15 sec at 13,000 rpm. The remaining ethanol was removed and the samples were left to air-dry for 15 min. The pellet was resuspended in 50 Bl of 5 mM Tris. After RNA isolation, cDNA was derived from each sample according to a previously described procedure (13).

PCR quantification of mRNA expression. Target cDNA sequences were amplified by quantitative PCR using a fluorescence-based real-time detection method (ABI PRISM 7900 Sequence Detection System, TaqMan®, Perkin-Elmer (PE) Applied Biosystems, Foster City, CA, USA) as previously described (19, 20). The PCR reaction

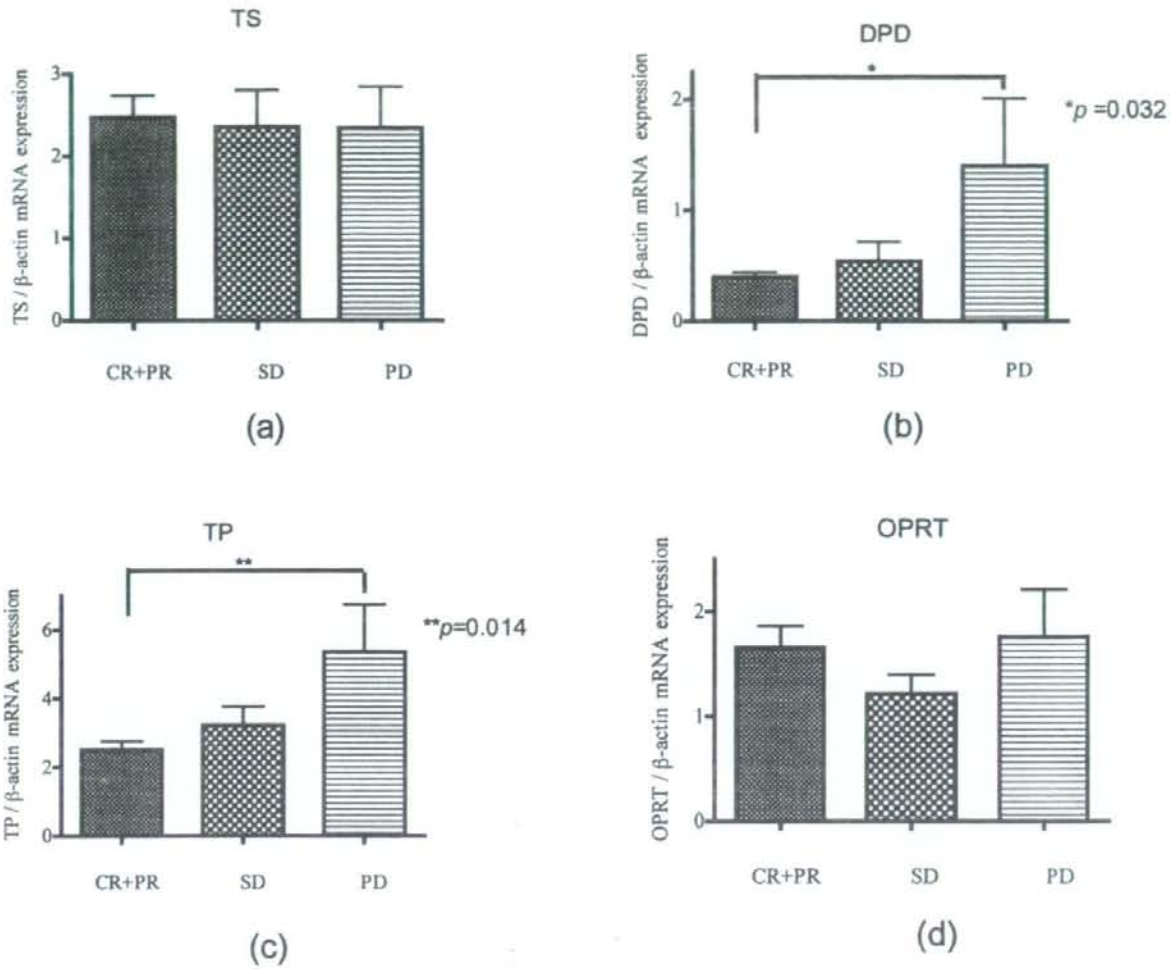

Figure 1. *mRNA expression ratio of thymidine synthase (TS) (a), dihydropyrimidine dehydrogenase (DPD) (b), thymidine phosphorylase (TP) (c), and oroteta phophoribosyl transferase (OPRT) (d) to β-actin in HAI-treated patients. CR=complete response, PR=partial response, SD=stable disease, PD=progressive disease.*

mixture (25 μL) contained 600 μmol/L of each primer, 200 nmol/L each of dATP, dCTP and dGTP, 400 μmol/L dUTP, 5.5 mmol/L $MgCl_2$, and 1x TaqMan buffer A containing a reference dye (all reagents were supplied by Applied Biosystems). The primers and probes sequences used were as follows: TS primers: GCCTCGGTGTGCCTTTCA and CCCGTGATGTGCGCAAT, probe 6FAM - TCGCCAGCTACGCCCTGCTCA; DPD primer: AGGACGCAAGGAGGGTTTG and GTCCGCCGAGTCCTTA CTGA, probe 6FAM - CAGTGCCTACAGTCTCGAGTCTG CCAGTG; TP primers: CCTGCGGACGGAATCCT and GCTG TGATGAGTGGCAGGCT, probe 6FAM - CAGCCAGAGATG TGACAGCCACCGT; OPRT primers: TAGTGTTTTGGAAA CTGTTGAGGTT and CTTGCCTCCCTGCTCTCTGT, probe 6FAM - TGGCATCAGTGACCTTCAAGCCCTCCT; β-actin primers: TGAGCGCGGCTACAGCTT and TCCTTAATGTCA CGCACGATTT, probe 6FAM - ACCACCACGGCCGAGCGG. PCR was performed at 50°C for 10 sec and 95°C for 10 min, followed by 42 cycles at 95°C for 15 sec and 60°C for 1 min. Gene expression values (relative mRNA levels) are expressed as ratios (differences between the Ct values) between the gene of TS, DPD, TP or OPRT and an internal reference gene (β-actin). This reference gene provides a baseline measurement for the amount of RNA isolated from a specimen

Statistical analysis. Differences in the expression of TS, DPD, TP, and OPRT between the CR/PR group, SD and PD groups were determined by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Correlations between the mRNA levels of TS, DPD, TP and OPRT were assessed using Spearmans's rank correlation. A value of $p<0.05$ was considered statistically significant. GraphPad Prism version 4.0 for Macintosh was used for the analyses.

Results

Therapeutic response and survival of patients treated by HAI. A CR in 5 patients, PR in 15 patients, SD in 9 patients, and PD in 9 patients were found. The overall response rate was 52.6%. The overall MST was 29.1 months.

5FU-related gene expression in HAI-treated patients. DPD and TP expression was significantly higher in the PD than in the CR/PR group ($p=0.032$, $p=0.014$, respectively) (Figure 1). There was no significant difference in the expression of TS or OPRT between the 3 subgroups. MST was not related to the expression of TS, DPD, TP, or OPRT. The mRNA levels of DPD and TP showed a significant correlation ($r=0.76$, $p=0.0001$) (Figure 2).

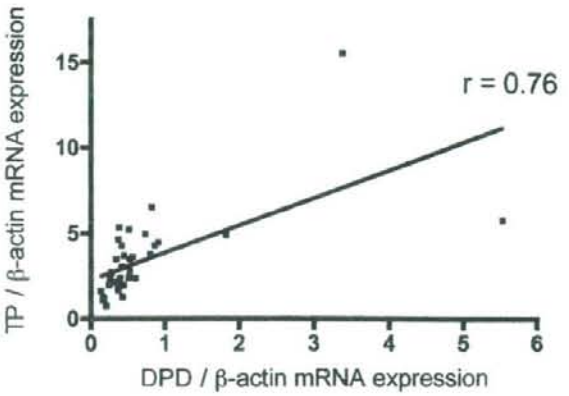

Figure 2. *Correlation between mRNA expression ratio of dihydropyrimidine dehydrogenase (DPD) and thymidine phosphorylase (TP) to β-actin in HAI-treated patients.*

Discussion

In the present HAI study, the expression of DPD and TP mRNAs were significantly lower in responders than in the PD group. Furthermore, DPD and TP expressions showed a significant correlation. DPD and/or TP were thus predictive factors for the therapeutic efficacy of HAI treatment. It has also previously been reported that DPD and TP expression in liver metastases of colorectal carcinomas correlated (21).

In the present study, TS expression did not vary significantly between the responding and non-responding groups. TS has been described as a key marker for predicting the therapeutic efficacy of 5FU-based systemic chemotherapy (9). The hepatic concentration of 5FU is much higher in patients treated by HAI than by systemic infusion. The mechanism of the antitumor effects of 5FU in HAI may be different from that in systemic chemotherapy and it may be more cytotoxic when administered by HAI than when given systemically. The antitumor effects of 5FU mainly involve two pathways: the inhibition of DNA synthesis and the inhibition of mRNA synthesis (22, 23). TS acts to catalyze the methylation of 2'-deoxyuridine-5'-monophosphate (dUMP) to 2'-deoxythymidine-5'-monophosphate (dTMP), which is an important process for DNA synthesis (22, 24). The 5FU metabolite 5-fluoro-2'-deoxyuridine-5'-monophosphate (FdUMP) forms a complex with TS and folic acid, which inhibits the de novo synthesis of dTMP from dUMP. In contrast, the pathway for inhibition of mRNA synthesis is not associated with TS. The 5-FU metabolite 5-fluorouridine-5'-triphosphate (FUTP) inhibits the synthesis of mRNA (25). The detailed mechanism by which FUTP inhibits mRNA synthesis has not been clearly defined. It is reported that bolus injection can be considered to be more effective with respect to RNA damage in tumor tissue (26, 27). As HAI in our study was performed with high dose 5FU in 5h, it is close to bolus injection more than continuous injection. The anti-tumor effect of HAI may be mainly due to the inhibition of mRNA. Physicians should consider CPT-11–based treatment for patients who show high TS gene expression levels prior to systemic chemotherapy generally (9, 10). However, according to our data, high TS gene expression would not be a limiting factor with HAI treatment.

DPD or TP, or both but not TS were demonstrated to be predictive factors of response to HAI treatment. No relationship between 5FU-related enzymes and survival time was found. Additional prospective studies will be required to determine whether the expression of these enzymes can be used to predict the prognosis of patients treated by HAI.

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5-Fluorouracil-related Gene Expression in Primary Sites and Hepatic Metastases of Colorectal Carcinomas

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Abstract. *The aim of this study was to investigate the correlation of the mRNA expressions of 5-fluorouracil (5FU)-related genes in the primary sites and liver metastases of colorectal carcinomas. Patients and Methods: Patients with liver metastases from colorectal carcinomas were included (n=43). The expression ratios to β-actin of mRNA of thymidine synthase (TS), dihydropyrimidine dehydrogenase (DPD), thymidine phosphorylase (TP) and oroteta phosphoribosyl transferase (OPRT) were measured in primary and liver metastases of colorectal carcinomas by laser-captured microdissection and real time PCR. Results: The ratios for the expression of TS, DPD, TP and OPRT mRNAs were significantly correlated between paired primary sites and liver metastases. The mRNA expression ratios of DPD and TP showed a significant correlation both in primary sites and in liver metastases. Conclusion: Enzymes of the primary colorectal carcinomas can be used in predicting the therapeutic efficacy of 5FU against liver metastases.*

Metastasis is the most important event that determines the prognosis of patients with advanced colorectal carcinoma (CRC). The liver is the most common target of metastases from CRCs. Surgical resection alone can result in a significant prolongation of survival in patients with favorable prognostic factors (1, 2). Systemic or regional chemotherapy regimens that include 5-fluorouracil (5FU) have been used to treat hepatic metastases in CRC patients when surgical resection cannot be performed (3-5).

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Key Words: Thymidine synthase, dihydropyrimidine dehydrogenase, thymidine phosphorylase, oroteta phosphoribosyl transferase, laser-captured microdissection.

5FU metabolism is regulated *in vivo* mainly by enzymes such as thymidine synthase (TS), dihydropyrimidine dehydrogenase (DPD), thymidine phosphorylase (TP), and oroteta phosphoribosyl transferase (OPRT). TS acts to catalyze the methylation of 2'-deoxyuridine-5'-monophosphate (dUMP) to 2'-deoxythymidine-5'-monophosphate (dTMP), which is an important process for DNA synthesis (6, 7). 5-Fluoro-2'-deoxyuridine-5'-monophosphate (FdUMP), a 5FU metabolite, forms a complex with TS and folic acid, which inhibits the *de novo* synthesis of dTMP from dUMP. The 5FU metabolite 5-fluorouridine-5'-triphosphate (FUTP) inhibits the synthesis of mRNA (8). The detailed mechanism by which FUTP inhibits mRNA synthesis has not been clearly defined. TP, also known as platelet-derived endothelial cell growth factor, plays an important role in the angiogenesis of carcinomas.

It has been reported that enzymes involved in 5FU metabolism, such as TS and DPD are important predictors of the therapeutic efficacy of 5FU (9, 10). It was reported that a high level of TP gene expression in CRC is associated with non-responsiveness to 5FU (11). However, in these studies, the enzymes which were reported to be responsible for the antitumor effects of 5FU were examined in primary sites of CRCs. The expression of enzymes involved in 5FU metabolism in metastatic site has not been examined. It is necessary to examine the relationship between the enzyme expression in primary and metastatic sites of CRCs.

The aim of this study was to investigate the correlation of the expression of TS, DPD, TP and OPRT mRNAs in primary sites and liver metastases of CRCs. The expression of TS, DPD, TP and OPRT genes was examined by a newly developed technique using laser-captured microdissection (LCM) combined with RNA extraction from paraffin-embedded specimens and RT-PCR (12-15). The LCM method made it possible to remove the contamination of adjacent normal tissue surrounding the carcinoma tissue and to purify the samples.

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Table I. *Patient characteristics.*

Characteristic	No. of patients
Gender	
Male	28
Female	15
Age (years; average)	62.0
Onset of liver metastasis	
Synchronous	27
Metachronous	16
Histology of primary colorectal carcinoma	
Well	11
Moderate	31
Poor	1
pTNM of primary colorectal carcinoma	
pT	
1	0
2	3
3	36
4	4
pN	
0	13
1	21
2	9
pM	
0	13
1	30

pTNM classification: a pathological classification for malignant tumors defined by UICC (International Union of Cancer).

Patients and Methods

Patients. Patients with synchronous or metachronous liver metastases originating from colorectal carcinomas were included (n=43). Their primary colorectal carcinomas and liver metastases were resected surgically. Patients who received preoperative irradiation were excluded. The patients characteristics are described in Table I. Written informed consent was obtained from all patients.

Microdissection. Four 10 μm-thick sections of the primary colorectal carcinomas and adjacent normal mucosa were prepared from the paraffin-embedded blocks. One 4 μm-thick section was prepared and stained with hematoxylin and eosin (HE). A representative formalin-fixed, paraffin-embedded (FFPE) tumor specimen was selected by a pathologist after examination of the HE-stained slides. Sections 10 μm in thickness were stained with neutral fast red to enable visualization of histology for LCM (PALM Microlaser Technologies AG, Munich, Germany), which was performed to ensure that only tumor cells were studied.

RNA extraction and cDNA synthesis. The RNA was isolated from the FFPE specimens using a novel, proprietary procedure (Response Genetics, Los Angeles, CA, USA) (9). The tissue samples to be extracted were placed in a 0.5 mL thin-walled tube containing 400 μl of 4 M dithiothreitol (DTT)-GITC/sarcosine (4 M guanidinium isothiocyanate, 50 mM Tris-HCl (pH 7.5), 25 mM EDTA) (Invitrogen, Carlsbad, CA, USA; No. 15577-018). The samples were homogenized and an additional 60 μl of GITC/sarc solution was added. They were heated at 92°C for 30 min and then transferred to a 2 mL centrifuge tube. Fifty microliters of 2 M sodium acetate (pH 4.0) were added, followed by 600 μl of freshly prepared phenol/chloroform/isoamyl alcohol (250:50:1). The tubes were vortexed for 15 s, placed on ice for 15 min and then centrifuged at 13,000 rpm for 8 min in a chilled (8°C) centrifuge. The upper aqueous phase was carefully removed and placed in a 1.5 mL centrifuge tube. Glycogen (10 μl) and 300-400 μl of isopropanol were added and the samples were vortexed for 10-15 s. The tubes were chilled at −20°C for 30-45 min to precipitate the RNA. The samples were then centrifuged at 13,000 rpm for 7 min in a centrifuge of 8°C. The supernatant was poured off and 500 μl of 75% ethanol were added. The tubes were again centrifuged at 13,000 rpm for 6 min in a chilled (8°C) centrifuge. The supernatant was then carefully poured off, so as not to disturb the RNA pellet, and the samples were quick-spun for another 15 s at 13,000 rpm. The remaining ethanol was removed and the samples were left to air-dry for 15 min. The pellet was resuspended in 50 μl of 5 mM Tris-HCl (pH 8.0). After RNA isolation, cDNA was derived from each sample according to a previously described procedure (12).

Table II. *Median mRNA expression ratio of thymidine synthase (TS), dihydropyrimidine dehydrogenase (DPD), thymidine phosphorylase (TP) and oroteta phosphoribosyl transferase (OPRT) in primary site and liver metastases.*

	Primary site	Liver metastases	*p*-value
TS	3.19 (0.73-8.35)	3.98 (0.34-18.5)	0.26
DPD	0.46 (0.09-1.41)	0.45 (0.08-1.44)	0.80
TP	3.16 (0.81-8.17)	2.72 (0.69-9.59)	**0.02**
OPRT	2.00 (0.63-4.24)	2.16 (0.45-5.51)	0.24

Expression ratio is shown as median value (range).

PCR quantification of mRNA expression. Target cDNA sequences were amplified by quantitative PCR using a fluorescence-based real-time detection method (ABI PRISM 7900 Sequence Detection System, TaqMan®; Perkin-Elmer (PE) Applied Biosystems, Foster City, CA, USA) as described elsewhere (16, 17). The PCR reaction mixture (25 μL) contained 600 μmol/L of each primer, 200 nmol/L each of dATP, dCTP and dGTP, 400 μmol/L dUTP, 5.5 mmol/L $MgCl_2$ and 1x TaqMan buffer A containing a reference dye (all reagents were supplied by Applied Biosystems). The primer and probe sequences used were as follows: TS primers: GCCTCGGTGTGCCTTTCA and CCCGTGATGTGCGCAAT, probe 6FAM–TCGCCAGCTACGCCCTGCTCA; DPD primers: AGGACGCAAGGAGGGTTTG and GTCCGCCGAGTCCTTAC TGA, probe 6FAM-CAGTGCCTACAGTCTCGAGTCTGCCAGTG; TP primers: CCTGCGGACGGAATCCT and GCTGTGATGAG TGGCAGGCT, probe 6FAM-CAGCCAGAGATGTGACAGC CACCGT; OPRT primers: TAGTGTTTTGGAAACTGTTGAGGTT and CTTGCCTCCCTGCTCTCTGT, probe 6FAM-TGGCATCA GTGACCTTCAAGCCCTCCT; β-actin primers: TGAGCGCG GCTACAGCTT and TCCTTAATGTCACGCACGATTT, probe 6FAM-ACCACCACGGCCGAGCGG.

PCR was performed at 50°C for 10 s and 95°C for 10 min, followed by 42 cycles at 95°C for 15 s and 60°C for 1 min. Gene expression values (relative mRNA levels) are expressed as ratios

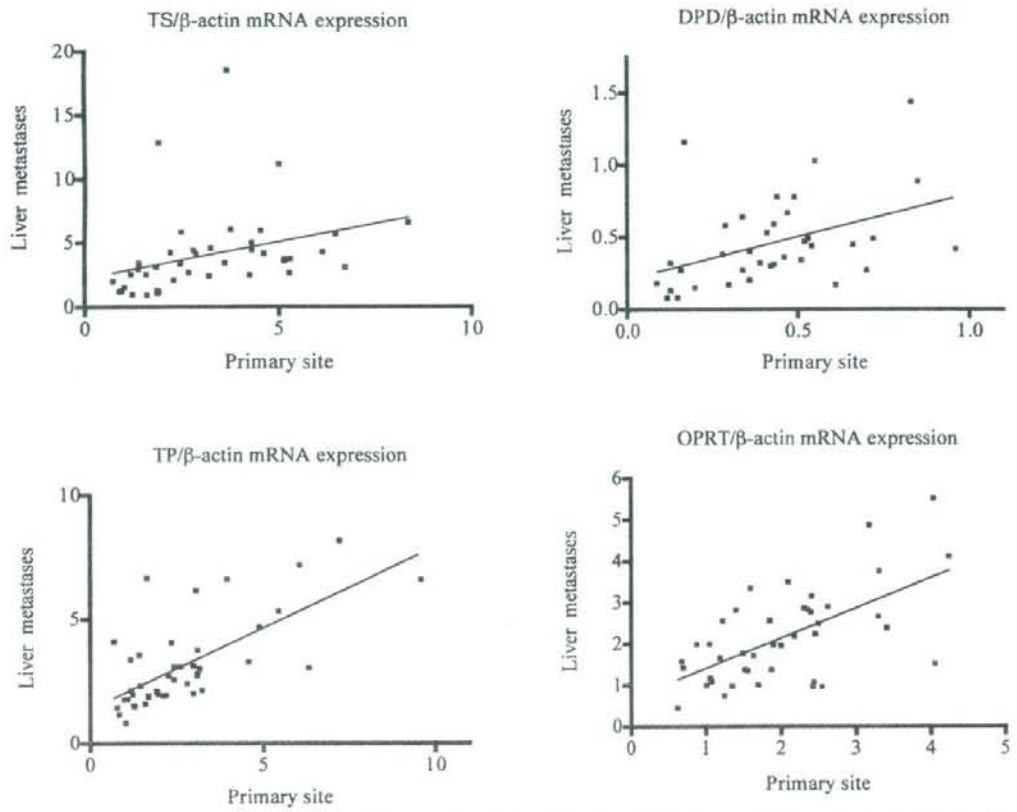

Figure 1. *Expression ratios of thymidine synthase (TS), dihydropyrimidine dehydrogenase (DPD), thymidine phosphorylase (TP) and oroteta phosphoribosyl transferase (OPRT) mRNA to β-actin in primary sites and liver metastases of colorectal carcinomas (TS, r=0.62, p<0.0001; DPD, r=0.50, p=0.0009; TP, r=0.65, p<0.0001; OPRT, r=0.50, p=0.0003).*

(differences between the Ct values) between the gene of TS, DPD, TP or OPRT and the internal reference gene β-actin. This reference gene provides a baseline measurement for the amount of RNA isolated from a specimen.

Statistical analysis. Differences in the mRNA expression ratios of TS, DPD, TP and OPRT in the primary sites and liver metastases were determined by the Wilcoxon signed rank test. Correlations between the mRNA levels of TS, DPD, TP and OPRT were assessed using Spearman's rank correlation. A value of $p<0.05$ was considered statistically significant. GraphPad Prism version 4.0 for Macintosh (San Diego, CA, USA) was used for the analyses.

Results

Gene expression levels in primary sites and liver metastases of CRCs. Median mRNA expression ratios of TS, DPD, TP and OPRT to β-actin are given in Table II. TP expression was significantly higher in primary sites than in their corresponding liver metastases. TS, DPD and OPRT did not differ significantly between primary sites and liver metastases.

Correlation of mRNA expression between primary sites and liver metastases of CRCs. The mRNA expression ratios of TS, DPD, TP and OPRT to β-actin in primary sites were significantly correlated to those in the liver metastases of CRCs (Figure 1).

Correlation between TS, DPD, TP and OPRT mRNA expressions in primary sites or liver metastases of CRCs. The mRNA expression of DPD and TP showed a significant correlation in both primary sites and in liver metastases (Figure 2).

Discussion

Our study demonstrated that the mRNA expression ratios of TS, DPD and OPRT in primary sites did not differ significantly from those in liver metastases. Only TP expression was significantly higher in primary sites than in liver metastases. There have been several studies which examined 5FU-related gene expression in primary and corresponding liver metastases from CRCs. However, their