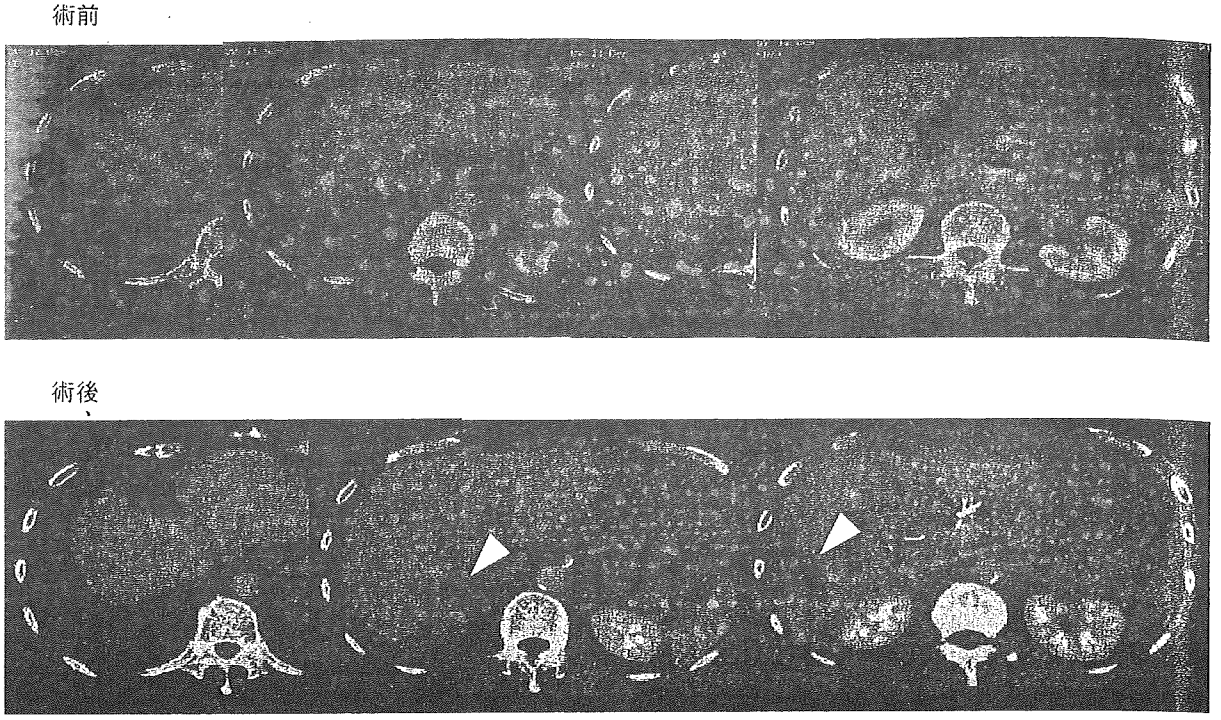

図1　肝切除にRFAを併用した症例のCT
S1, S3, S4の病巣に対し部分切除を行い，深部のS6とS7の病巣に対しRFAを施行した．

併用することが可能である．しかし，MCTやRFAなどの凝固療法では必ずしも組織学的な完全壊死が得られるとは限らず，また腫瘍の複数回穿刺は肝内転移や遠隔転移を誘発する危険性がある．

一般に肝切除の限界は正常肝の場合，残肝量が30%～40%を下回ると厳しいと言われている．切除不能肝転移のもう一つの治療戦略として熱凝固療法を併用し，残肝量を確保することによる切除率の向上が考えられる．そのためには熱凝固療法の局所制御能や熱凝固が与える腫瘍学的側面を把握することが必須である．本研究では切除不能両葉多発大腸癌肝転移に対する熱凝固療法併用の意義を検討する．さらに，化学療法先行＋熱凝固療法併用により切除可能となった症例を検討し，切除率向上のための化学療法＋肝熱凝固療法併用について展望する．

1 対象と方法

1994年から2006年までに教室で肝切除（凝固療法を含む）を施行した大腸癌肝転移137例を対象とした．内訳は肝切除単独95例，肝切除＋凝固療法27例，凝固療法単独15例であった．凝固療法はMicrowave coagulation therapy (MCT) 36病巣，radiofrequency ablation (RFA) 52病巣の計88病巣に対しすべて開腹下または開胸下に施行した．原則として凝固療法は，3 cmより小さい主要な脈管に接しない病巣で，切除量が多くなると予測される深い病巣に対して行った(図1)．また，肝外病変を有し同時切除では過大侵襲となる症例や肝切除に耐術不能な症例に対して凝固療法を併用または単独で施行した．凝固療法の局所制御能を評価し，両葉多発肝転移症例を対象に無再発生存率および生存率を切除単独群と比較した．さらに，肝無再発生存率と生存率を規定する因子の多変量解析を行った．

2 結　果

1 凝固療法の対象病変

凝固療法の対象病変を表1に示す．凝固単独例では，1病巣が7例，2病巣が4例，3病巣が1例と4病巣以上が3例であった．肝切除併用例では，それぞれ12例，9例，2例および4例であった．平均腫瘍径は単独例が20 mm，併用例

表1　肝凝固療法の対象病変

	凝固施行病巣数				腫瘍径 (mm)
	1	2	3	>3	
凝固療法単独 (n=15)	7	4	1	3	20±15 (5～40)
肝切除併用 (n=27)	12	9	2	4	18±10 (10～32)
計	19	13	3	7	19±11 (5～40)

では18 mmであった．

2）凝固療法の局所制御能

42例88病巣（MCT：36病巣，RFA：52病巣）をCT検査にて追跡した．CT検査は術後1週後と術後3年までは6ヶ月毎，3年以降は年に1度，さらにCEAなど腫瘍マーカーの上昇や再発が疑われる場合は随時施行した．治療部位または治療部位に連続する再発を治療部位再発と定義した．治療部位再発は，42例中4例（9.5%）に認められ，MCT：3/36病巣（8.3%）とRFA：2/52病巣（3.8%）であり，全体で5/88病巣(5.7%)，局所制御能は94%と高率あった．

3）治療法と転移状況

転移状況と治療法との関連をみてみると，肝切除単独は単発が59例，片葉多発が18例，両葉多発が18例と単発例が多いのに対し，肝切除＋凝固療法は両葉多発が23例と最も多く適応されていた．凝固療法単独はそれぞれ7例，3例および5例であった（表2）．

表2　治療法と転移状況

治療法	転移巣		
	単発	片葉多発	両葉多発
肝切除単独	59例	18例	18例
肝切除＋凝固療法	0例	4例	23例
凝固療法単独	7例	3例	5例
計	66例	25例	46例

表3　凝固療法の有無別症例の背景（両葉多発例）

治療	年齢 (歳)	性別 (M/F)	肝転移巣		肝外病変あり (%)
			数*	腫瘍径 (mm)	
肝切除単独 (n=18)	62±8	14/4	3.8±1.9	48±29	2 (11%)
凝固療法 (n=28)	56±13	19/9	5.1±2.7	29±10	3 (11%)

* $p<0.05$

4）両葉多発例の背景

前述の治療法別転移状況の違いから凝固療法の遠隔成績を両葉多発例に限って検討した．肝切除単独例と凝固療法例の背景を表3に示す．凝固療法例の平均転移個数は5.1±2.7個と肝切除単独例の3.8±1.9個に比べ有意に多かった．また，有意差はないものの切除単独例は年齢が高く，腫瘍径は大きい傾向を認めた．

5）無再発生存率

治療後の無再発生存率は凝固療法例が肝切除単独例に比べ有意（$p=0.047$）に低率であった．さらに肝無再発生存率ではその差（$p=0.011$）が顕著であった（図2）．

6）生存率

凝固療法例と肝切除単独例には有意な生存率の差異は認めなかった（$p=0.245$）（図3）．

7）予後因子の多変量解析

肝無再発生存期間および生存期間に影響する因子の多変量解析を行った．共変量として年齢，性別，転移個数，転移腫瘍径，肝外病変の有無，肝転移の時期，肝動注の有無，凝固療法の有無，原発巣リンパ節転移の有無，原発巣組織型（高分化とそれ以外）を検討した．肝無再発生存期間では独立した予後良好因子として肝動注と異時性肝転移が予後不良因子として原発巣リンパ節転移(+)と腫瘍径≧50 mmが選択された．生存期間では原発巣リンパ節転移（+）と肝外病変（+）の2因子のみが独立した予後不良因子であった（表4）．

8）化学療法により肝切除可能となった症例

2007年より切除不能な肝転移巣が両葉に10個以上の5症例に対し，化学療法を施行した．5例の転移個数は19.2±3.9個，腫瘍径は3.9±1.0

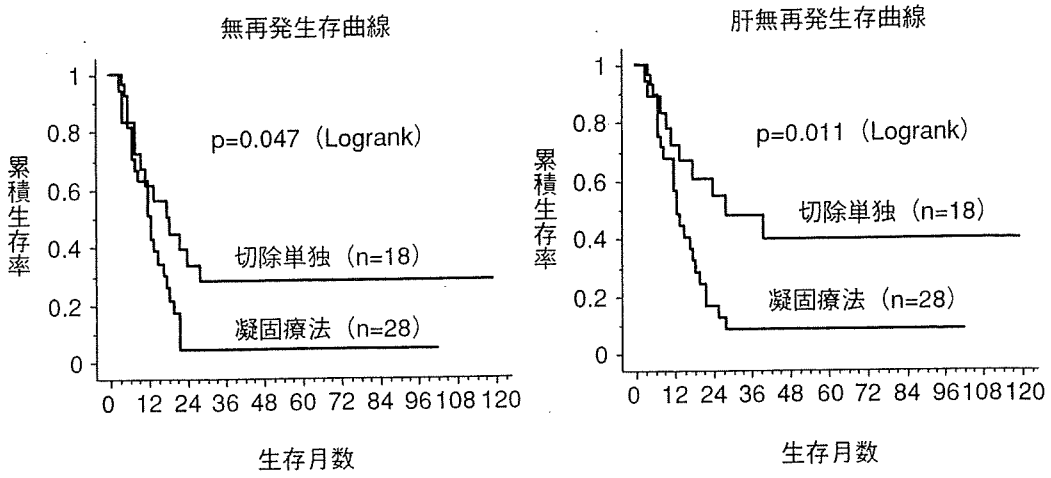

図2　肝凝固療法例と肝切除単独例の無再発生存曲線―両葉多発例―

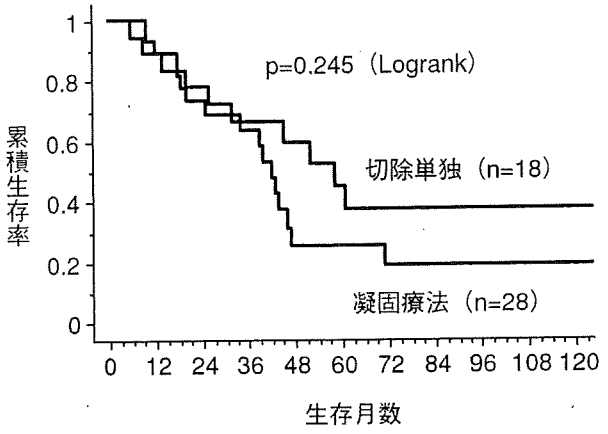

図3　肝凝固療法例と肝切除単独例の生存曲線―両葉多発例―

表4　予後因子の多変量解析

因　子	p値	95%信頼区間	ハザード比
（肝無再発生存期間）			
術後肝動注（＋）	0.006	0.069～0.644	0.211
異時性転移	0.014	0.051～0.710	0.190
原発巣リンパ節転移（＋）	0.012	1.394～14.900	4.558
腫瘍径≧50 mm	0.027	1.216～23.944	5.395
（全生存期間）			
原発巣リンパ節転移（＋）	0.015	1.328～14.483	4.385
肝外病変（＋）	0.046	1.026～13.699	3.745

mmであった．化学療法のレジメンはIRIS（CPT-11/S-1）が3例，IRIS/FOLFOX4が1例，mFOLFOX6が1例であった．いずれも化学療法の直接効果はPRが得られた．このうち57歳の男性（症例1）と51歳（症例2）の男性の2例が熱凝固（RFA）併用の肝切除が可能となった．切除不能同時性多発肝転移を伴う直腸癌に対し，原発巣切除後症例1にはIRIS，症例2にはIRISからFOLFOX4の化学療法を行い，症例1ではRFAを10病変と部分切除を3病変に行った．さらに残肝に再発を認め，再RFAを6病変に施行した．症例2では3病変にRFAを併用し，10病変の部分切除を施行した（図4）．2008年7月現在，肝に3病変の再発を認めており，化学療法を施行中で今後再切除（RFA）の予定である．

3 考　察

凝固療法の治療部位再発率は5.7%（MCT: 8.3%, RFA: 3.8%）と低率であった．この結果は肝部分切除に匹敵するものであり，凝固療法の適応を厳格にした結果によるものと考えられる．すなわち，凝固療法はすべて開腹下または開胸下に原則として3 cm以下の主要脈管に接しない小病変に対して施行した．MCTの局所制御能について経皮アプローチによる30 mmより小さい単発転移巣の15例中13例に完全凝固が得られ，治療部位再発は認められなかった（観察期間9から37カ月）ことが報告されている[4]．一方，RFAの局所制御能については当初50%を越す局所再発率が報告された[5,6]．しかしその後，肝細胞癌を含む検討ではあるがRFAの治療部位再発は

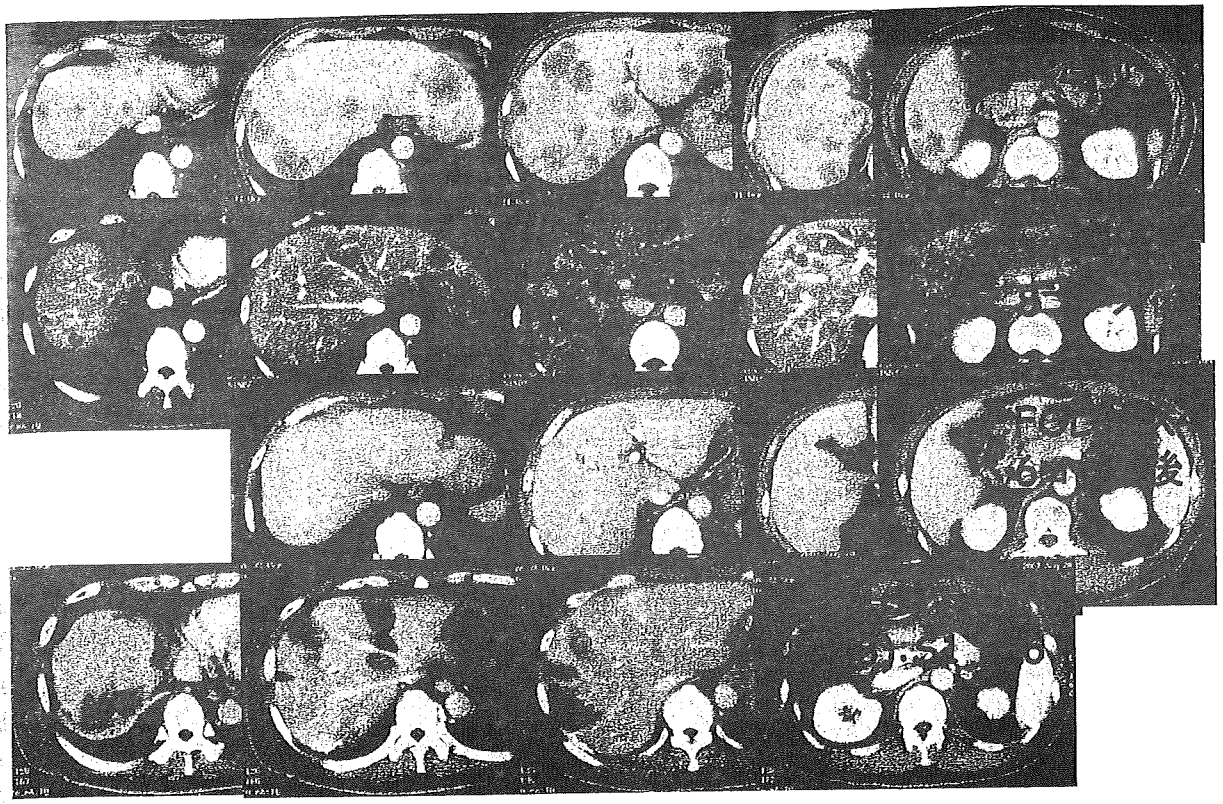

図4　化学療法により切除＋RFAが可能となった症例のCT

169病巣中3病巣（1.8%）と良好な局所制御能が報告された[7]．良好な局所制御率の理由について，経皮アプローチは3個未満の30 mmより小さい転移巣に限り，他の腫瘍は開腹下に施行したことが指摘されている．さらに開腹下のRFAでは，血流の冷却効果による不完全凝固を懸念してpringle-maneuver techniqueを用いている．また，大腸癌肝転移88症例227病巣に対する開腹下RFAの局所再発率は5.7%と64症例99病巣に対する肝部分切除の7.1%と遜色ないことが報告されている[8]．やはり，腫瘍径が30 mm以上や主要脈管に接する病巣は治療部位再発と関連があることが報告されている．一方，経皮的RFAでは40%の依然高い再発率が報告[9]されている．但し，その再発は腫瘍径と関連が深く，25 mm以下では78%，26から40 mmまでは47%，40 mm以上では32%の局所制御率であった．経皮的RFAは開腹下のRFAと比較して治療部位再発の有意な要因であることが示されている[10]．これらの結果は，MCTやRFAなど凝固療法は厳格な適応や適切なアプローチを選択することで部分切除に匹敵する局所制御能を有することを示唆している．

最近，肝切除に併施した開腹下のRFA症例では凝固部位の再発は低率で，しかも転移個数や腫瘍径が同等であるにも関わらず肝切除単独例に比べ残肝再発が高率であることが報告されている[11]．筆者らの肝無再発生存率の結果も転移個数は凝固療法例が多いものの同様な傾向であった．これらは凝固療法が残肝再発を誘発する可能性を示唆しているが，多変量解析から凝固療法自体は残肝再発の独立した因子ではなく，凝固療法例の低い肝無再発生存率は転移個数や分布など他の要因が複雑に関与している結果と推測される．

このように凝固療法が肝部分切除と同等の遠隔成績をもたらすならば肝の深い部位に存在する小転移巣に凝固療法を併用することで切除不能な両葉多発肝転移症例が切除可能となる．さらに，肝外病変に対する過大な侵襲を伴う場合や心・肺合併症を有するpoor risk症例では時として肝切除を断念せざるを得ない．このような症例でも凝固療法を適応することにより肝切除が可能となり，肝外病変に対しても姑息的治療ではなく根治的治療を選択することができる．

凝固療法を併用しても切除不能な場合，化学療法の腫瘍縮小効果を期待する．化学療法を先行することによる切除の可能性として，①主要脈管に浸潤する病変の縮小による脈管切除の回避，②腫瘍の縮小による凝固療法の適応（腫瘍径が大きい転移巣は不完全凝固となるため），③病変消失による肝切除デザインの再構築，などが考えられ，いずれも残肝量を確保することが可能となる．Adamら[3]は，切除不能肝転移症例701例に対しFOLFOXを施行した結果，95例（14%）が切除可能になったと報告している．加えて，切除が可能になった症例の5年生存率は30%を越え良好であった．

転移個数が多いため切除不能となる症例では，病変の消失が切除可能の条件となる．しかし，化学療法により画像上消失した病変の多くは組織学的には腫瘍の遺残が報告されている[12]．筆者らの2例も残肝に再発を認めており，消失病変の再増殖の可能性が高い．しかし，これらは再度切除やRFAが可能であり，化学療法でたとえ組織学的腫瘍消失が得られなくても分割切除（staged operation）の概念で根治治療が期待できる．

まとめ

大腸癌肝転移の治療成績を向上させる一つの手段は，肝切除率を高めることである．両葉多発転移例は技術的に切除不能となることが多く，凝固療法を併用することにより切除が可能となる．大腸癌肝転移に対する凝固療法の局所制御能は，技術の習得と適応さえ誤らなければ肝部分切除に匹敵するとはいえ，現状では原則として完全切除不能例がこれら凝固療法の適応と考える．この場合，凝固療法では根治性が劣ると考えられる大きな腫瘍や主要な脈管に近接する病巣はできるだけ切除することが望ましい．肝切除も凝固療法も不能な症例には全身化学療法を先行し，効果が得られれば切除や凝固の可能性を検討する．肝切除にきわめて近い局所制御能を有し，かつ低侵襲性である凝固療法の大腸癌肝転移治療における役割は今後益々高まり，さらなる治療成績の向上に貢献するものと思われる．

文　献

1) Rodgers MS, McCall JL: Surgery for colorectal liver metastases with hepatic lymph-node involvement: a systematic review. *Br J Surg* **87**: 1142–1155, 2000
2) Kato T, Yasui K, Hirai T, et al: Analysis of prognostic factors for 763 cases recorded at 18 institutions. *Dis Colon Rectum* **46** (Suppl): S22–31, 2003
3) Adam R, Avisar E, Ariche A, et al: Five-year survival following hepatic resection after neoadjuvant therapy for nonresectable colorectal. *Ann Surg Oncol* **8**: 347–353, 2001
4) Seki T, Wakabayashi M, Nakagawa T, et al: Percutaneous microwave coagulation therapy for solitary metastatic liver tumor from colorectal cancer: a pilot clinical study. *Am J Gastroenterol* **94**: 322–327, 1999
5) Solbiati L, Ierace T, Goldberg SN, et al: Percutaneous US-guided radiofrequency tissue ablation of liver metastases: treatment and follow-up in 16 patients. *Radiology* **202**: 195–203, 1997
6) Mazziotti A, Grazi GL, Gardini A, et al: An appraisal of percutaneous treatment of liver metastases. *Liver Transpl Surg* **4**: 271–275, 1998
7) Curley SA, Izzo F, Delrio P, et al: Radiofrequency ablation of unresectable primary and metastatic hepatic malignancies: results in 123 patients. *Ann Surg* **230**: 1–8, 1999
8) Elias D, Baton O, Sideris L, et al: Local recurrences after intraoperative radiofrequency ablation of liver metastases: a comparative study with anatomic and wedge resections. *Ann Surg Oncol* **11**: 500–505, 2004
9) Solbiati L, Livraghi T, Goldberg SN, et al: Percutaneous radio-frequency ablation of hepatic metastases from colorectal cancer: long-term results in 117 patients. *Radiology* **221**: 159–166, 2001
10) Amersi FF, McElrath-Garza A, Ahmad A, et al: Long-term survival after radiofrequency ablation of complex unresectable liver tumors. *Arch Surg* 141: 581–587, 2006
11) Abdalla EK, Vauthey JN, Ellis LM, et al: Recurrence and outcomes following hepatic resection, radiofrequency ablation, and combined resection/ablation for colorectal liver metastases. *Ann Surg* **239**: 818–825, 2004
12) Benoist S, Brouquet A, Penna C, et al: Complete response of colorectal liver metastases after chemotherapy: does it mean cure? *J Clin Oncol* 24: 3939–3945, 2006

日本大腸肛門病会誌 62：77-81，2009

原　著 I

大腸癌の同時性，異時性肝・肺転移に対する外科治療の成績と問題点

村上　英嗣　緒方　裕　赤木　由人
石橋　生哉　笹冨　輝男　白水　和雄
久留米大学外科

大腸癌の肝・肺同時転移例や肝または肺切除を行った後に肝あるいは肺転移をきたし切除を行った例（以下，二臓器目転移例）に対する外科的治療の適応や成績は不明な点も多い．1990年から2007年までに肝切除および肺切除を行った大腸癌肝・肺転移症例24例を対象とし大腸癌肝・肺転移に対する外科治療の適応と問題点を検討した．肝切除先行が19例，肝・肺同時切除が5例で，肺切除先行例は認めなかった．二臓器目切除以降の生存期間の中央値は39.5カ月であった．残肝または残肺再発を15例（62.5%）に認め，このうち再切除を施行できたのは残肺再発例で9例中6例（66.7%），再切除後の生存期間は平均28カ月であった．残肝再発例，残肝・残肺再発例についてはいずれも制御困難であった．大腸癌肝・肺転移例に対する外科治療後の再発は高率であった．しかし，肝および肺切除後の生存期間の中央値は39.5カ月で，長期生存が得られている症例もあり積極的切除の意義は大きいと考えられた．

索引用語：大腸癌，肝・肺転移，外科治療

はじめに

近年，大腸癌に対する外科治療の進歩により治癒切除率や根治性は向上しているが，予後をさらに改善するためには遠隔転移に対する治療成績の向上が重要である．大腸癌の主たる遠隔転移臓器は肝および肺であり，肝切除や肺切除の良好な成績が報告されている[1-4]．しかし，肝・肺同時転移例や肝切除または肺切除の既往ある症例で，その後肝転移や肺転移をきたした症例に対する外科的治療の適応と成績は十分に検討されてはいない．そこで，我々は当科における大腸癌肝・肺転移症例を対象に外科治療の適応と問題点を検討した．

対象と方法

1990年から2007年までに当科で肝切除および肺切除を行った大腸癌肝・肺転移症例24例を対象とし，同時性あるいは異時性に肝と肺を切除（以下，二臓器目切除と略）した後の生存率，残肝および残肺再発率，再発症例を検討し大腸癌肝・肺転移に対する外科治療の適応と問題点を検討した．累積生存率はKaplan-Meier法で算出し，Logrank検定にて判定した．2群間の比較はt検定を用い，いずれも$p<0.05$をもって有意差ありとした．なお，臨床病理学的事項の記載は，大腸癌取り扱い規約第6版[5]に従った．

結　果

全24例の内訳は男性9例・女性15例で，二臓器目切除時の年齢は平均59歳であった．原発巣の局在は結腸11例・直腸13例で，病理組織学的背景は組織型は高分化腺癌12例・中分化腺癌12例，深達度はssまたはa1：9例，seまたはa2：13例，si：2例，リンパ節転移度はn0：11例，n1：13例であった．リンパ管侵襲度ではly0：5例，ly1：11例，ly2：5例，ly3：3例，静脈侵襲度はv0：3例，v1：18例，v2：3例であった（表1）．

肝転移巣の背景はH1：18例，H2：4例，H3：2例で，転移個数は平均2.6個，腫瘍最大径は平均31 mmであった．術式では11例に系統的切除が施行された．切除時期の内分けでは同時性転移13例，異時性転移11例であった．肺転移巣の属性背景は片葉20例・両葉4例，転移個数は平均1.7個，最大径平均18mmであった．術式は部分切除が18例，肺葉切除

表1　患者背景

年齢（mean±SD，歳）		59±11
性別（M/F）		9/15
原発巣	部位（Colon/Rectum）	11/13
	組織型（wel/mod）	12/12
	深達度（ss, a1/se, a2/si）	9/13/2
	n（0/1）	11/13
	ly（0/1/2/3）	5/11/5/3
	v（0/1/2）	3/18/3

表2　肝転移巣と肺転移巣の背景

肝転移巣		肺転移巣	
H1/H2/H3	18/4/2	片葉/両葉	20/4
個数（mean±SD，個）	2.6±2.1	個数（mean±SD，個）	1.7±1.1
最大径（mean±SD，mm）	31±16	最大径（mean±SD，mm）	18±8
系統的切除	13	切除（部/葉）	18/6
時性（同/異）	13/11	時性（同/異）	4/20

が6例であり，手術時期の内分けは同時性転移4例，異時性転移20例であった（表2）．肝または肺の切除順をみると，肝切除先行が19例，肝・肺同時切除が5例で，肺切除先行例は認めなかった（表3）．原発巣切除から肝切除までの期間は平均8カ月，肺切除までの期間は平均26カ月で両群間に有意差を認めた．肝切除から肺切除までの期間は平均16カ月で，この間に肺以外の再発が5例に認められた．内訳は肝3例，腹膜1例，鼠径リンパ節1例でいずれも外科的治療にて制御可能であった（表4）．

二臓器目切除以降の生存期間の中央値は39.5カ月で，10年以上の長期生存例もみられた．無再発生存期間の中央値は10.5カ月で，再発を18例（75%）に認めた（図1）．

二臓器目切除以降の再発部位の内訳は残肺9例，残肝4例，残肝・残肺2例，脳1例，腹膜1例，局所1例で（表5）残肝再発と残肺再発の無再発生存期間の比較では両群間に差は認めなかった（図2）．これらの再発部位に対する再切除は残肺再発に対してのみ6例に施行され再切除率は66.7%，再切除後生存期間は平均28カ月であった．さらに，再肺切除後の残肺再発を3例に認めたが2例に対して再々肺切除を施行した．残肝再発はいずれも多発転移のため再肝切除例はなかったが，肝動注による補助療法にて

表3　肝切除および肺切除の時性

	症例数（例）	原発巣切除からの期間（mean±SD，mo.）
肝切除先行	19	9±13
肺切除先行	0	
肝・肺同時切除	5	6±7

CRが得られた例が1例であった．これらの再発後生存期間は平均23カ月であった．残肝・残肺再発も多発転移のため切除不能であり，再発後生存期間は平均8カ月であった．また，切除可能な肝・肺以外の転移巣はなくいずれも制御困難であり，これらの無再発生存期間は平均14カ月，再発後生存期間は平均6カ月であった（表5）．

考　察

大腸癌肝転移や肺転移に対する外科的切除の成績について，初回治癒的肝切除または肺切除後の5年生存率は30～60%と報告されている[1-4]．さらに，肝転移と肺転移を同時にもしくは異時的に認める肝・肺転移症例に対する切除例の5年生存率は11～44%と報告されている[6-8]．多くは肝切除を行った後に肺転移をきたし肺切除が行われているが，Ambiruらは6例中1例（直腸癌症例）[9]，Lehnertらは17例中1例は肺切除先行例であったと報告している[10]．自検例では肺転移（切除）先行例は認めなかった．同時性肺転移4例中2例が下部直腸症例であった．下部直腸症例では転移巣および再発巣の検索の際には肺病巣にも特に注意を要すると思われた．原発巣切除から一臓器目切除までの期間は平均8.2カ月と短く，自検例では同時性転移症例が54.2%と半数以上を占めた．一臓器目切除から二臓器目切除までの期間は平均16.3カ月で，他の報告の平均18～23.5カ月とほぼ同等であった．二臓器目切除後の生存の中央値は39.5カ月と良好であった．他の報告でも中央値34～48カ月と良好である[9-11]．二臓器目切除後の再発について詳細に検討した報告は少ない．自検例では二臓器目切除後の無再発期間の中央値は10.5カ月，再発率は残肝再発と残肺再発で62.5%，他の部位と合わせると75%と高率であった．さらに残肝再発形式はいずれも多発転移であり，肝動注でCRが1例にみられたが再切除例はなかった．今後oxaliplatin，irinotecanやbevacizumabなどによる

表4　肝切除および肺切除の時期

原発巣切除から肝切除までの期間	8±12（mean±SD, 月）	*
原発巣切除から肺切除までの期間	26±24（mean±SD, 月）	
一臓器目切除から二臓器目切除までの期間	16±16（mean±SD, 月）	
【この間の再発　5例（肝3例　腹膜1例　鼠径1例）】		

*$p < 0.05$

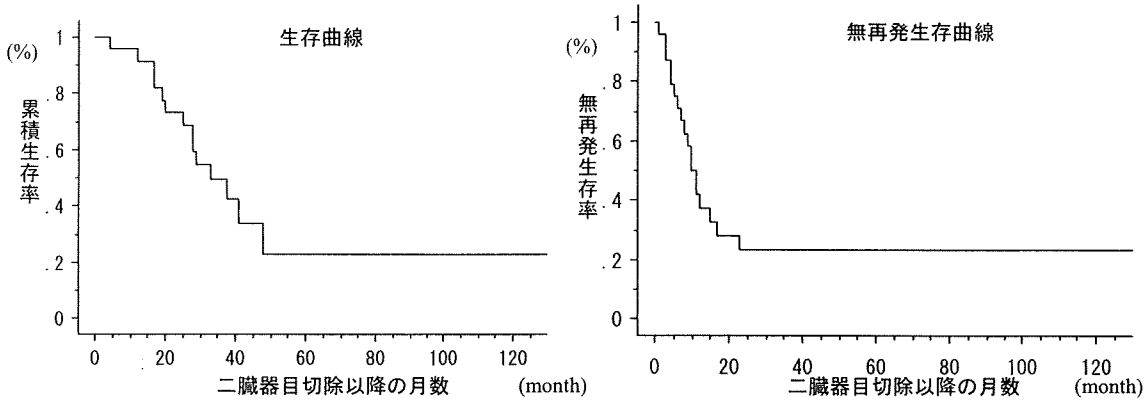

図1　累積生存率および累積健存率

表5　二臓器目切除以降の初再発部位の内訳

初再発部位	症例数（例）	DFS（mean, mo.）	再発後生存（mean, mo.）	再切除（例）	再々切除（例）
肺	9	7	25	6（66.7%）	2
肝	4	8	23	—	
肝・肺	2	9	8	—	
局所	1				
腹膜	1	14	6	—	
脳	1				
計	18（75%）				

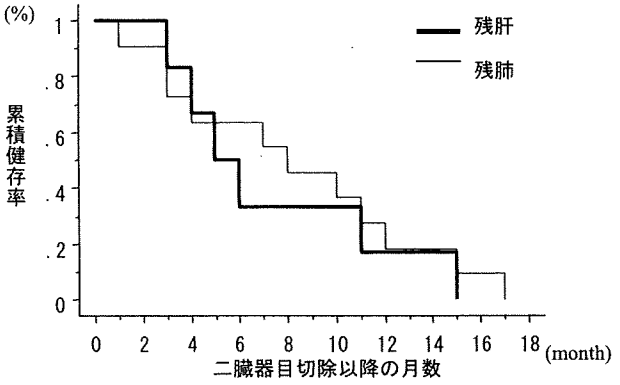

図2　残肝再発および残肺再発の無再発生存率　p = 0.668

全身化学療法により切除可能例が増えることが予測される．しかし，化学療法により画像上または肉眼上転移巣が消失しても病理学的には高率で残存するとの報告もあり[12]，化学療法著効例に対する切除の適応や時期については検討の余地があると思われる．また，残肝再発予防策として近年では術後肝動注による残肝再発予防で予後の改善が得られたとする報告も散見されてはいるがその意義についてはいまだ確立されていない[13-15]．自検例では7例(29.2%)に肝切除後の肝動注を施行したが，非施行群に比べ残肝再発を有意に抑制するという結果は得られなかった．残肺再発に対しては再切除率66.7%，再切除後の生存期間は平均28カ月と良好な結果が得られた．さらに再々発に対しての切除も2例に行われた．Ikeらは大腸癌術後の肺転移単独切除と肝転移切除

後に肺転移をきたして切除を行った症例を比較し，生存に差はないとしている[11]．さらに，既往歴としての肝転移に対する肝切除は肺転移切除後の予後因子にはならないとの報告もあり，肺転移巣に対する外科的治療の意義は大きいと考える[16-19]．二臓器目切除後，肝と肺に同時再発した症例および肝肺以外に再発した症例に対しては再切除を行えず，化学療法などを施行したがいずれも制御困難であり再発後の生存期間も短かった．

結　語

大腸癌の肝と肺の二臓器転移例に対する外科切除後の経過をみると，再発率は高く再切除率も低率であった．しかし，肝・肺切除後の生存期間の中央値は39.5カ月で長期生存が得られている症例もあった．さらに，肺に再々発する症例では再切除により長期生存も期待できる例もあった．

文　献

1) 安井健三，清水泰博，平井　孝ほか：大腸癌肝転移の外科治療　他施設による根治的肝切除の検討．癌と化学療法　31：690-694, 2004
2) 横井佳博，鈴木昌八，中村　達：大腸癌肝転移に対する外科治療．癌と化学療法　29：848-855, 2002
3) 緒方　裕，的野敬子，林　明宏ほか：大腸癌肺転移に対する肺切除の遠隔成績．日本臨床外科学会雑誌　62：2110-2115, 2001
4) 藤田秀人，藪下和久，吉岡伊作ほか：大腸癌肺転移症例の手術治療成績．日本消化器外科学会雑誌　35：144-150, 2002
5) 大腸癌研究会編：大腸癌取り扱い規約．第6版．金原出版，東京，1998
6) 石津寛之，近藤征文，益子博幸ほか：大腸癌肝転移切除例における肺転移巣切除の臨床意義に関する検討．日本大腸肛門病学会雑誌　57：43-48, 2004
7) Kobayashi K, Kawamura M, Ishihara T, et al: Surgical treatment for both pulmonary and hepatic metastases from colorectal cancer. J Thorac Cardiovasc Surg 118 (6): 1090-1096, 1999
8) Hamy A, Baron O, Bennouna J, et al: Resection of hepatic and pulmonary metastases in patients with colorectal cancer. Am J Clin Oncol 24 (6): 607-609, 2001
9) Ambiru S, Miyazaki M, Ito H, et al: Resection of hepatic and pulmonary metastases in patients with colorectal carcinoma. Cancer 82: 274-278, 1998
10) Lehnert T, Knaebel HP, Duck M, et al: Sequential hepatic and pulmonary resections for metastatic colorectal cancer. British Journal of Surgery 86: 241-243, 1999
11) Ike H, Shimada H, Togo S, et al: Sequential resection of lung metastases following partial hepatectomy for colorectal cancer. British Journal of Surgery 89: 1164-1168, 2002
12) Benoist S, Brouquet A, Penna C, et al: Complete response of colorectal liver metastases after chemotherapy: Does it mean cure? J Clin Oncol 24: 3939-3945, 2006
13) 辻　寧重，浜田弘巳，木村　純ほか：大腸癌肝転移切除後予防的肝動注の共同研究．癌と化学療法　26：1694-1697, 1999
14) 東野　健，大里浩樹，蓮池康徳ほか：大腸癌肝転移に対する動注化学療法の有用性と限界．癌と化学療法　26：1741-1746, 1999
15) 吉田　晋，裕　彰一，近藤浩史ほか：大腸癌肝転移切除後補助療法としての肝動注の検討．癌と化学療法　33：1845-1847, 2006
16) Watanabe I, Arai T, Ono M, et al: Prognostic factors in resection of pulmonary metastasis from colorectal cancer. Br J Surg 90: 1436-1440, 2003
17) Girard P, Ducreux M, Baldeyrou P, et al: Surgery for lung metastases from colorectal cancer. J Clin Oncol 14: 2047-2053, 1996
18) Yano T, Hara N, Ichinose Y, et al: Results of pulmonary resection of metastatic colorectal cancer and its application. J Thorac Cardiovasc Surg 106: 875-879, 1993
19) McAfee MK, Allen MS, Trastek VF, et al: Colorectal lung metastases: results of surgical excision. Ann Thrac Surg 53: 780-786, 1992

Resection of Hepatic and Pulmonary Metastases in Colorectal Carcinoma

Hidetsugu Murakami, Yutaka Ogata, Yoshito Akagi, Nobuya Ishibashi,
Teruo Sasatomi and Kazuo Shirouzu
Department of Surgery, Kurume University School of Medicine

We have retrospectively reviewed the clinical significance of the resection of hepatic and pulmonary metastases in 24 patients with colorectal carcinoma between 1990 and 2007. Previous hepatic resection was performed in 19 patients and combined hepatic and pulmonary resection was performed in 5 patients. No cases were found in patients with previous pulmonary resection. The median survival time after resection of the second organ was 39.5 months. Of the 24 patients, 15 patients (62.5%) showed hepatic or pulmonary recurrence. There were 9 patients who showed pulmonary recurrence, of which 6 (66.7%) underwent further pulmonary resection with a mean survival time after the second resection of 28 months. There were 6 patients who showed recurrence of hepatic metastasis, and none underwent any further resection. Most other other cases of recurrence were uncontrollable. Surgical treatment for hepatic and pulmonary metastases with colorectal carcinoma tended to show a high recurrence rate, but the median survival time after resection of the second organ was 39.5 months and several cases showed a very much longer survival time. Accordingly, it is concluded that surgical treatment of hepatic and pulmonary metastases shows clinical benefit even in the long term.

（2008年 5 月 16 日受付）
（2008年 7 月 24 日受理）

ANTICANCER RESEARCH *28*: 389-394 (2008)

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

SHINICHI SAMESHIMA[1], SHIGERU TOMOZAWA[1], HIROYUKI HORIKOSHI[2], KENTA MOTEGI[3], ISAO HIRAYAMA[1], SHINICHIRO KOKETSU[1], TOSHIYUKI OKADA[1], MASARU KOJIMA[4], YOUICHI KON[3] and TOSHIO SAWADA[1]

Departments of [1]Surgery, [2]Diagnostic Radiology, [3]Gastroenterology, and [4]Pathology, Gunma Cancer Center, Ota, Gunma, Japan

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).

Correspondence to: Shinichi Sameshima, MD, Ph.D., Department of Surgery, Gunma Cancer Center, 617-1 Takabayashi-nishi, Ota, Gunma, Japan 373-8550. Tel: +81 276 38 0771, Fax: +81 276 38 8386, e-mail: shinsame@gunma-cc.jp

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

0250-7005/2008 $2.00+.40

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

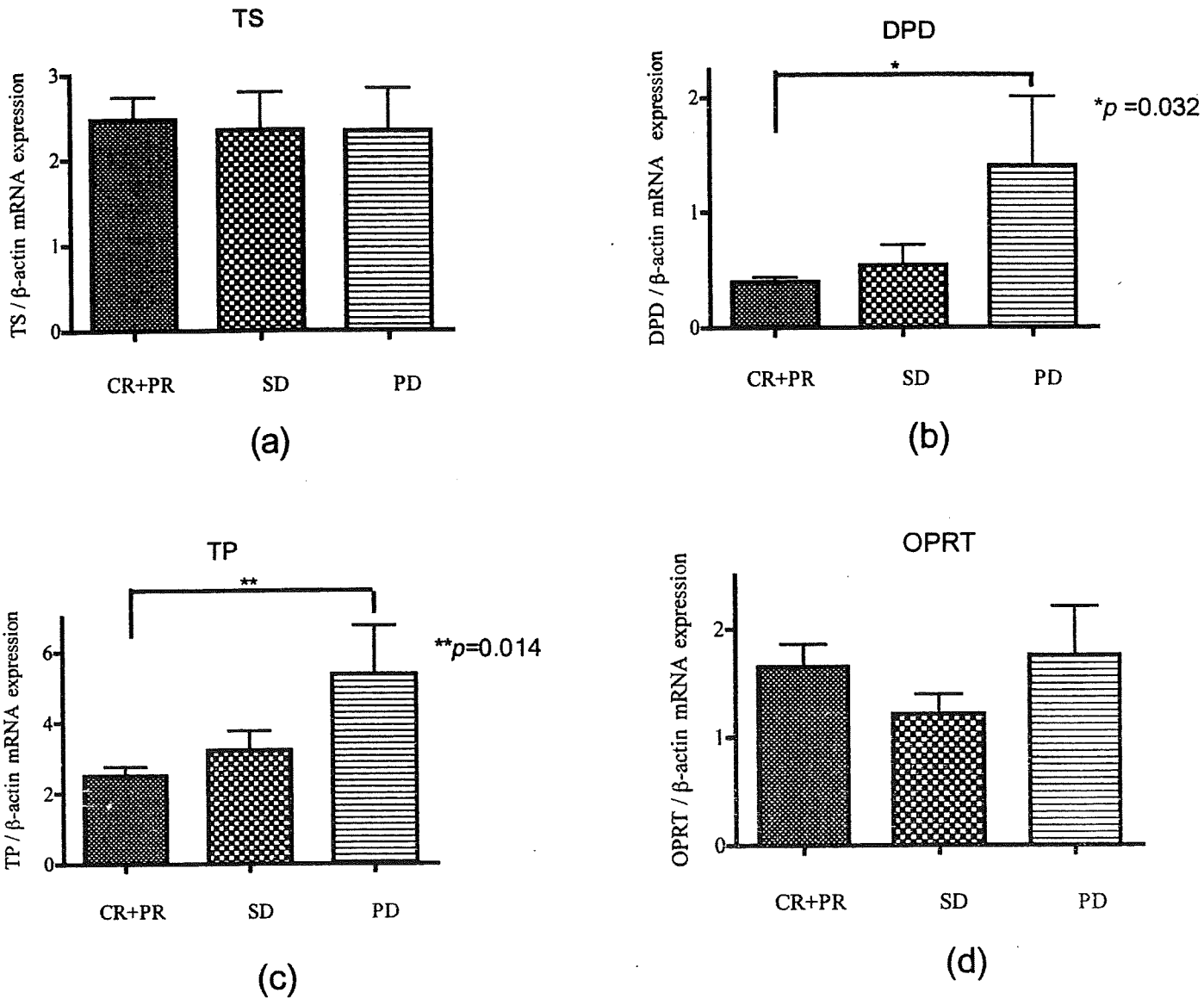

Figure 1. *mRNA expression ratio of thymidine synthase (TS) (a), dihydropyrimidine dehydrogenase (DPD) (b), thymidine phosphorylase (TP) (c), and oroteta phophoribosyl transferase (OPRT) (d) to β-actin in HAI-treated patients. CR=complete response, PR=partial response, SD=stable disease, PD=progressive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

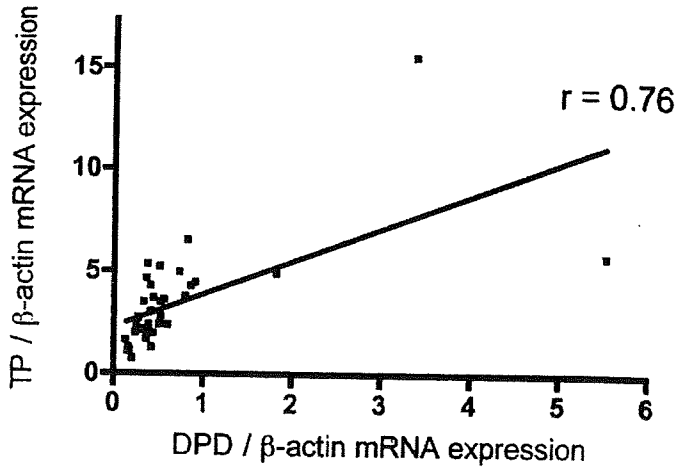

Figure 2. *Correlation between mRNA expression ratio of dihydropyrimidine dehydrogenase (DPD) and thymidine phosphorylase (TP) to β-actin in HAI-treated patients.*

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.

Acknowledgements

The authors are indebted to Professor J. Patrick Barron of the International Medical Communications Center of Tokyo Medical University for his review of this manuscript.

References

1 Gayowski TJ, Iwatsuki S, Madariaga JR, Selby R, Todo S, Irish W and Starzl TE: Experience in hepatic resection for metastatic colorectal cancer: analysis of clinical and pathologic risk factors. Surgery *116*: 703-710, 1994.

2 Scheele J, Stang R, Altendorf-Hofmann A and Paul M: Resection of colorectal liver metastases. World J Surg *19*: 59-71, 1995.

3 de Gramont A, Figer A, Seymour M, Homerin M, Hmissi A, Cassidy J, Boni C, Cortes-Funes H, Cervantes A, Freyer G, Papamichael D, Le Bail N, Louvet C, Hendler D, de Braud F, Wilson C, Morvan F and Bonetti A: Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. J Clin Oncol *18*: 2938-2947, 2000.

4 Goldberg RM, Sargent DJ, Morton RF, Fuchs CS, Ramanathan RK, Williamson SK, Findlay BP, Pitot HC and Alberts SR: A randomized controlled trial of fluorouracil plus leucovorin, irinotecan, and oxaliplatin combinations in patients with previously untreated metastatic colorectal cancer. J Clin Oncol *22*: 23-30, 2004.
5 Martin JK Jr, O'Connell MJ, Wieand HS, Fitzgibbons RJ Jr, Mailliard JA, Rubin J, Nagorney DM, Tschetter LK and Krook JE: Intra-arterial floxuridine *vs.* systemic fluorouracil for hepatic metastases from colorectal cancer. A randomized trial. Arch Surg *125*: 1022-1027, 1990
6 Kelly RJ, Kemeny NE and Leonard GD: Current strategies using hepatic arterial infusion chemotherapy for the treatment of colorectal cancer. Clin Colorectal Cancer *5*: 166-174, 2005.
7 Kemeny NE, Niedzwiecki D, Hollis DR, Lenz HJ, Warren RS, Naughton MJ, Weeks JC, Sigurdson ER, Herndon JE, 2nd, Zhang C and Mayer RJ: Hepatic arterial infusion versus systemic therapy for hepatic metastases from colorectal cancer: a randomized trial of efficacy, quality of life, and molecular markers (CALGB 9481). J Clin Oncol *24*: 1395-1403, 2006.
8 Sameshima S, Horikoshi H, Motegi K, Tomozawa S, Hirayama I, Saito T and Sawada T: Outcomes of hepatic artery infusion therapy for hepatic metastases from colorectal carcinoma after radiological placement of infusion catheters. Eur J Surg Oncol *33*: 741-745, 2007.
9 Salonga D, Danenberg KD, Johnson M, Metzger R, Groshen S, Tsao-Wei DD, Lenz HJ, Leichman CG, Leichman L, Diasio RB and Danenberg PV: Colorectal tumors responding to 5-fluorouracil have low gene expression levels of dihydropyrimidine dehydrogenase, thymidylate synthase, and thymidine phosphorylase. Clin Cancer Res *6*: 1322-1327, 2000.
10 Inoue T, Hibi K, Nakayama G, Komatsu Y, Fukuoka T, Kodera Y, Ito K, Akiyama S and Nakao A: Expression level of thymidylate synthase is a good predictor of chemosensitivity to 5-fluorouracil in colorectal cancer. J Gastroenterol *40*: 143-147, 2005.
11 Meropol NJ, Gold PJ, Diasio RB, Andria M, Dhami M, Godfrey T, Kovatich AJ, Lund KA, Mitchell E and Schwarting R: Thymidine phosphorylase expression is associated with response to capecitabine plus irinotecan in patients with metastatic colorectal cancer. J Clin Oncol *24*: 4069-4077, 2006.
12 Metzger R, Danenberg K, Leichman CG, Salonga D, Schwartz EL, Wadler S, Lenz HJ, Groshen S, Leichman L and Danenberg PV: High basal level gene expression of thymidine phosphorylase (platelet-derived endothelial cell growth factor) in colorectal tumors is associated with nonresponse to 5-fluorouracil. Clin Cancer Res *4*: 2371-2376, 1998.
13 Lord RV, Salonga D, Danenberg KD, Peters JH, DeMeester TR, Park JM, Johansson J, Skinner KA, Chandrasoma P, DeMeester SR, Bremner CG, Tsai PI and Danenberg PV: Telomerase reverse transcriptase expression is increased early in the Barrett's metaplasia, dysplasia, adenocarcinoma sequence. J Gastrointest Surg *4*: 135-142, 2000.
14 Farrugia DC, Ford HE, Cunningham D, Danenberg KD, Danenberg PV, Brabender J, McVicar AD, Aherne GW, Hardcastle A, McCarthy K and Jackman AL: Thymidylate synthase expression in advanced colorectal cancer predicts for response to raltitrexed. Clin Cancer Res *9*: 792-801, 2003.
15 Emmert-Buck MR, Bonner RF, Smith PD, Chuaqui RF, Zhuang Z, Goldstein SR, Weiss RA and Liotta LA: Laser capture microdissection. Science *274*: 998-1001, 1996.
16 Ichikawa W, Takahashi T, Suto K, Nihei Z, Shirota Y, Shimizu M, Sasaki Y and Hirayama R: Thymidylate synthase and dihydropyrimidine dehydrogenase gene expression in relation to differentiation of gastric cancer. Int J Cancer *112*: 967-973, 2004.
17 Tanaka T, Arai Y, Inaba Y, Matsueda K, Aramaki T, Takeuchi Y and Kichikawa K: Radiologic placement of side-hole catheter with tip fixation for hepatic arterial infusion chemotherapy. J Vasc Interv Radiol *14*: 63-68, 2003.
18 Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom AT, Christian MC and Gwyther SG: New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst *92*: 205-216, 2000.
19 Heid CA, Stevens J, Livak KJ and Williams PM: Real time quantitative PCR. Genome Res *6*: 986-994, 1996.
20 Gibson UE, Heid CA and Williams PM: A novel method for real time quantitative RT-PCR. Genome Res *6*: 995-1001, 1996.
21 Kuramochi H, Hayashi K, Uchida K, Miyakura S, Shimizu D, Vallbohmer D, Park S, Danenberg KD, Takasaki K and Danenberg PV: 5-Fluorouracil-related gene expression levels in primary colorectal cancer and corresponding liver metastasis. Int J Cancer *119*: 522-526, 2006.
22 Langenbach RJ, Danenberg PV and Heidelberger C: Thymidylate synthetase: mechanism of inhibition by 5-fluoro-2'-deoxyuridylate. Biochem Biophys Res Commun *48*: 1565-1571, 1972.
23 Matsuoka H, Ueo H, Sugimachi K and Akiyoshi T: Preliminary evidence that incorporation of 5-fluorouracil into RNA correlates with antitumor response. Cancer Invest *10*: 265-269, 1992.
24 Peters GJ, van der Wilt CL, van Triest B, Codacci-Pisanelli G, Johnston PG, van Groeningen CJ and Pinedo HM: Thymidylate synthase and drug resistance. Eur J Cancer *31A*: 1299-1305, 1995.
25 Roobol C, De Dobbeleer GB and Bernheim JL: 5-Fluorouracil and 5-fluoro-2'-deoxyuridine follow different metabolic pathways in the induction of cell lethality in L1210 leukaemia. Br J Cancer *49*: 739-744, 1984.
26 Aschele C, Sobrero A, Faderan MA and Bertino JR: Novel mechanism(s) of resistance to 5-fluorouracil in human colon cancer (HCT-8) sublines following exposure to two different clinically relevant dose schedules. Cancer Res *52*: 1855-1864, 1992.
27 Hoshino S, Yamashita Y, Maekawa T and Shirakusa T: Effects on DNA and RNA after the administration of two different schedules of 5-fluorouracil in colorectal cancer patients. Cancer Chemother Pharmacol *56*: 648-652, 2005.

Received September 25, 2007
Revised December 12, 2007
Accepted December 18, 2007

ANTICANCER RESEARCH 28: 1477-1482 (2008)

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

SHINICHI SAMESHIMA[1], SHIGERU TOMOZAWA[1], MASARU KOJIMA[2], SHINICHIRO KOKETSU[1], KENTA MOTEGI[3], HIROYUKI HORIKOSHI[4], TOSHIYUKI OKADA[1], YOUICHI KON[3] and TOSHIO SAWADA[1]

Departments of [1]Surgery, [2]Pathology, [3]Gastroenterology and [4]Diagnostic Radiology, Gunma Cancer Center, Ota, Gunma, Japan

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).

Correspondence to: Shinichi Sameshima, MD, Ph.D., Department of Surgery, Gunma Cancer Center, 617-1 Takabayashi-nishi, Ota, Gunma, 373-8550, Japan. Tel: +81 276 380771, Fax: +81 276 388386, e-mail: shinsame@gunma-cc.jp

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.

0250-7005/2008 $2.00+.40

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

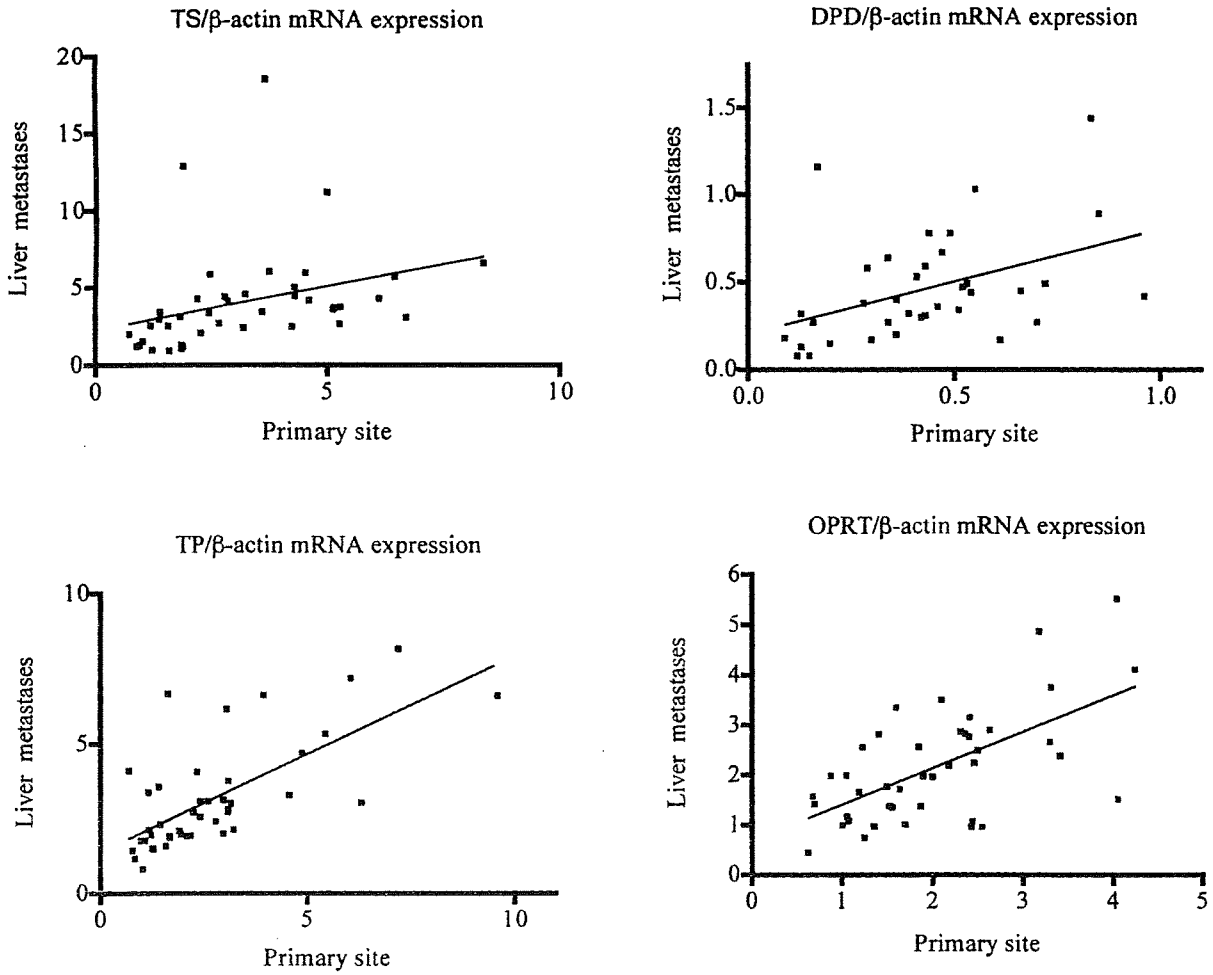

Figure 1. *Expression ratios of thymidine synthase (TS), dihydropyrimidine dehydrogenase (DPD), thymidine phosphorylase (TP) and oroteta phosphoribosyl transferase (OPRT) mRNA to β-actin in primary sites and liver metastases of colorectal carcinomas (TS, r=0.62, p<0.0001; DPD, r=0.50, p=0.0009; TP, r=0.65,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

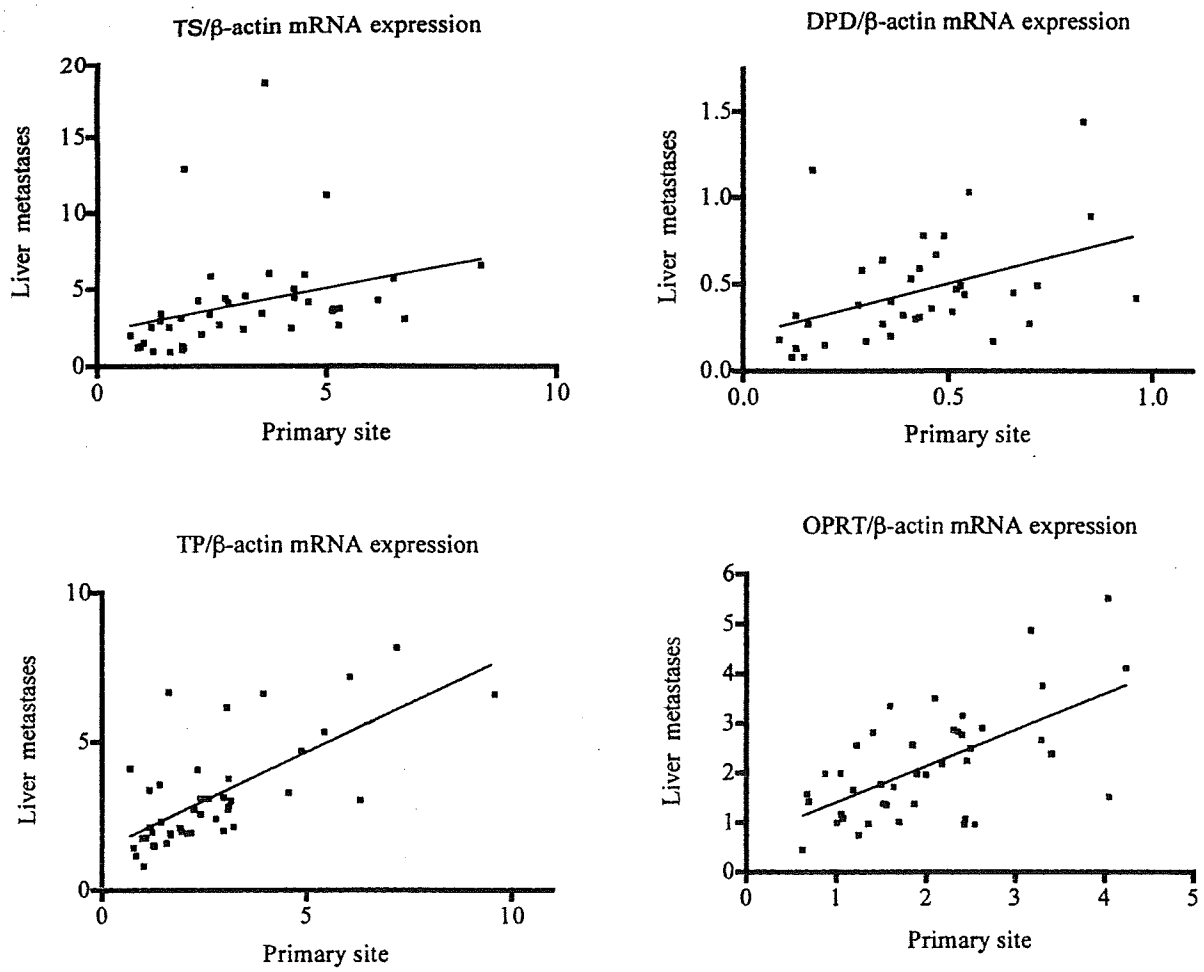

Figure 1. *Expression ratios of thymidine synthase (TS), dihydropyrimidine dehydrogenase (DPD), thymidine phosphorylase (TP) and oroteta phosphoribosyl transferase (OPRT) mRNA to β-actin in primary sites and liver metastases of colorectal carcinomas (TS, r=0.62, p<0.0001; DPD, r=0.50, p=0.0009; TP, r=0.65,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

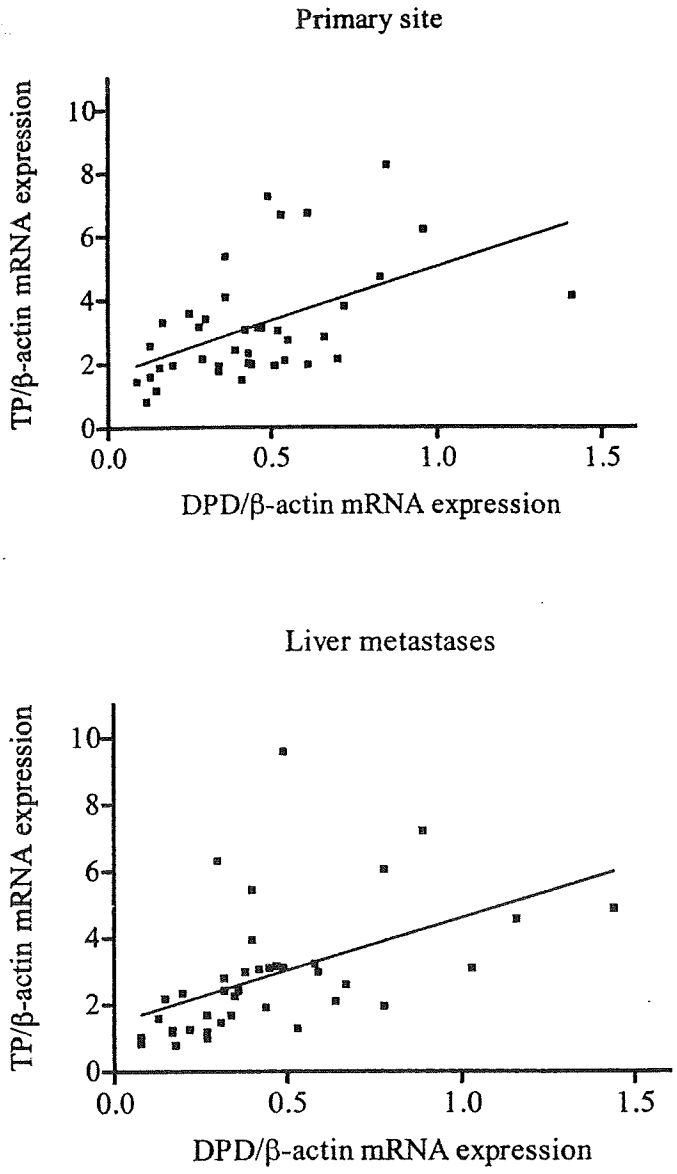

Figure 2. *Relationship between DPD and TP expression ratios to β-actin in primary sites (r=0.54, p=0.0004) and liver metastases (r=0.68, p<0.0001) of colorectal carcinomas.*

results were controversial. Inokuchi *et al.* reported that the DPD, OPRT and TP mRNA levels were significantly higher in liver metastases than in primary tumors and that TS mRNA levels did not differ significantly (18), however, they did not use the LCM method to purify tissue. DPD, TP and OPRT were reported to show higher expression levels in normal liver tissue than in the liver metastases (19). The contamination of normal liver tissue with metastatic liver tissue may have affected their results. Kuramochi *et al.* reported no significant differences between median mRNA expression levels of TS, DPD, TP and OPRT in primary carcinoma and those in corresponding liver metastases (19). Their TP expression levels were lower in the metastatic liver site than in the primary sites, but not significantly.

We also demonstrated that the mRNA expression levels of TS, DPD, TP and OPRT in liver metastases were significantly correlated to those in primary sites of CRCs. Kuramochi *et al.*, using the LCM method, reported a significant correlation for TS mRNA expression between primary carcinomas and corresponding liver metastases and no correlation for DPD, TP or OPRT (19). Their method was similar to ours. These different results may be due to the sample condition or sample size.

Among the four genes that were studied, a significant correlation was observed between DPD and TP both in the primary sites and the liver metastases. Inokuchi *et al.* and Kuramochi *et al.* reported similar results (18, 19). Mori *et al.* reported a positive correlation between DPD and TP protein levels in colorectal, pancreatic, esophageal, bladder, cervical, hepatic and gastric carcinomas (20). It was reported that DPD and TP gene expression in CRCs were associated with tumor progression (21, 22). A high level of TP gene expression is reported to be associated with non-responsiveness to 5FU (11). TP is supposed to play as an important role in tumor progression and 5FU sensitivity as TS or DPD.

The expression of 5FU-related enzymes has been used to predict the therapeutic efficacy and survival of 5FU-treated patients. It has been reported that the clinical response and survival rates in response to 5FU-based chemotherapy for patients with CRC are related to the expression of TS, DPD and TP (9, 23). We also reported that the expression levels of DPD and TP mRNAs in primary CRCs was significantly predictive of the therapeutic response to hepatic arterial infusion of 5FU (24). The expression of these enzymes is important for guiding the rational selection of chemotherapeutic regimens. Physicians should consider using a regimen that includes irinotecan (CPT-11) for patients who show a high expression of TS, DPD and TP in their carcinomas. In this study, we showed the positive correlation of gene expression of TS, DPD, TP and OPRT between primary carcinomas and liver metastases. Analysis of primary carcinomas can be used to predict the gene expression level in the liver metastases. Our results confirm the idea that the levels of gene expression in primary carcinomas can be used for directing the strategies of the chemotherapy against metastases. We have not examined the gene expressions in extrahepatic metastases such as lung metastases. Further studies are required for metastases from other organs.

References

1 Gayowski TJ, Iwatsuki S, Madariaga JR, Selby R, Todo S, Irish W and Starzl TE: Experience in hepatic resection for metastatic colorectal cancer: analysis of clinical and pathologic risk factors. Surgery *116*: 703-710, 1994.

2 Scheele J, Stang R, Altendorf-Hofmann A and Paul M: Resection of colorectal liver metastases. World J Surg *19*: 59-71, 1995.