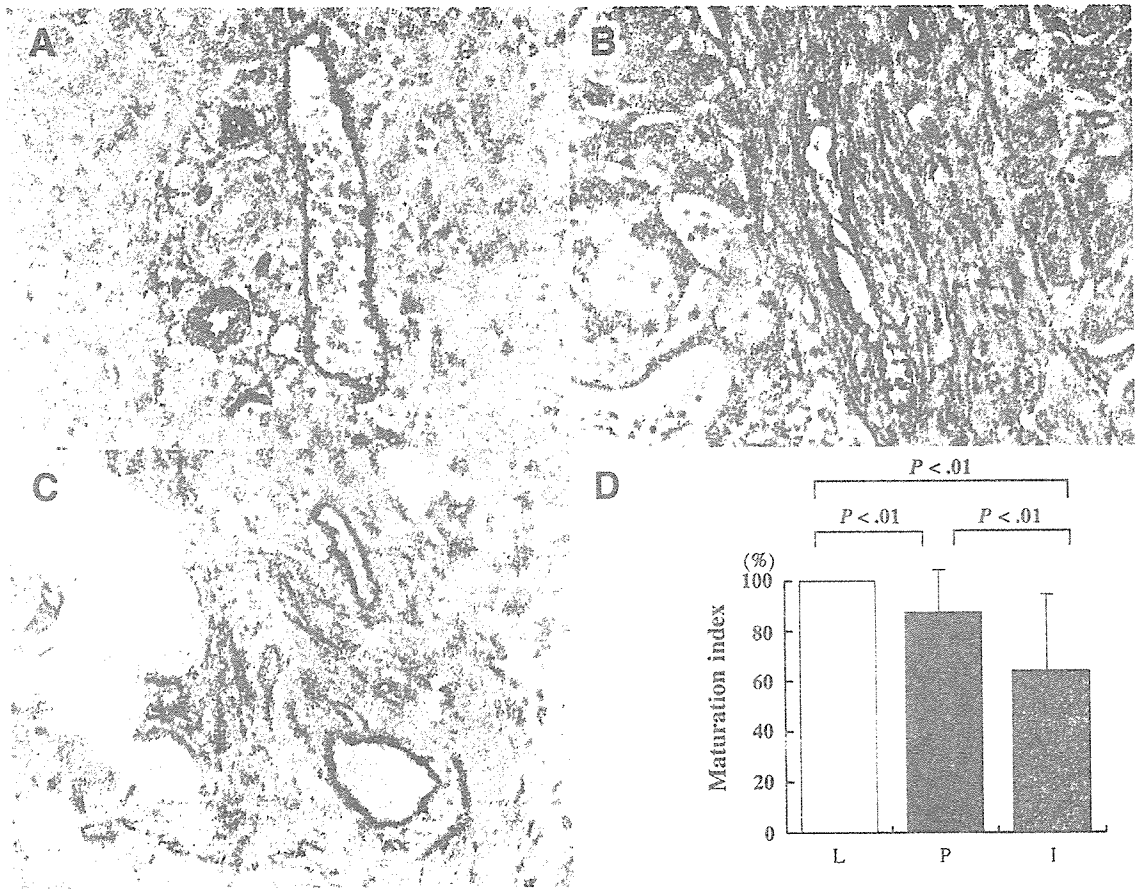

Fig. 10. Vessel maturation. ECs and PESCs were stained on the same section with anti-CD31 antibody and anti-α-SMA antibody, respectively, using the double-staining technique. Vessels were characterized by CD31 staining for ECs (brown). α-SMA staining (pink) was a marker for PESCs. (A) Mature vessels, such as the portal vein and the hepatic artery, were virtually surrounded by PESCs. (B) Vessels in the peripheral region of the metastatic CRC lesion in the liver partially lacked α-SMA expression derived from PESCs. A small number of α-SMA-positive myofibroblastlike cells also were present in the stroma. (C) In the intermediate region, vessels were surrounded by fewer PESCs. (D) The vessel maturation index was 100% ± 0% in adjacent liver tissue, 87.6% ± 20.1% in the tumor periphery, and 64.2% ± 28.1% in the intermediate region: all differences were significant ($P < .01$). L, liver region adjacent to the metastatic CRC lesion; P, periphery of the metastatic CRC lesion; I, intermediate region of the metastatic CRC lesion. (Original magnification ×200.)

Of several angiogenic factors (*e.g.*, beta fibroblast growth factor and platelet-derived endothelial cell growth factor), VEGF is the best-characterized proangiogenic agent that is known to be involved in the development of metastatic CRC in the liver.[30-32] The usefulness of anti-VEGF agents currently is being tested in clinical trials.[33] By using VEGF as a control in the current study, the relevance of Ang-2 in metastatic CRC in the liver was made more evident. The RNA expression pattern of *ANG2*, but not *ANG1*, was very similar to that of *VEGF* (Fig. 6), and we found a significant correlation between expression of *ANG2* RNA and expression of *VEGF* RNA in each tumor region (Fig. 7). Previous studies have demonstrated that Ang-2 promotes angiogenesis synergistically with VEGF in several *in vivo* models[10,13] and that coexpression of Ang-2 and VEGF often is observed in gastric cancer, glioma, thyroid cancer, and lung cancer.[16-18,21] Together, Ang-2 and VEGF may participate in tumor-associated angiogenesis in liver metastases.

Although RT-PCR assays provide data on gene expression at the RNA level, biologic function is carried out by proteins. Thus, insight into the expression of the angiogenesis-related proteins themselves would be desirable. There is evidence that expression of *VEGF* messenger RNA (mRNA) is well correlated with VEGF protein expression in human hepatocellular carcinoma.[34,35] It also has been reported that *ANG1*, *ANG2*, and *TIE2* RNA expression data were concordant with the corresponding protein expression data in human hepatocellular carcinoma and in rheumatoid arthritis.[35,36] We also found good agreement between RNA and protein expression data for the four angiogenesis-related molecules investigated (Fig. 8). Furthermore, immunohistochemical analysis provided confirmatory results regarding Ang-2 and VEGF expression in metastatic CRC. These findings suggest that expression of these proteins may be regulated at a transcriptional level.

The current study raised the question of why RNA expression of *ANG2* and *VEGF* increased going from the

tumor periphery to the intermediate region when vascular density was lower in the intermediate region compared with the periphery. To explain this paradox, we posited that insufficient blood supply in the intermediate region might stimulate production of the angiogenic factors in question, given that *VEGF* is a putative hypoxia-inducible gene.[37] The relatively hypoxic environment in the intermediate portion as compared with the periphery was verified by RT-PCR analysis of another hypoxia-inducible gene, *GLUT1*[38] (glucose transporter gene-1) (Ogawa M et al., unpublished data, 2003). In support of this hypothesis, there is evidence that hypoxia can induce *ANG2* expression in vascular ECs and glioma cells.[21,39–41]

Another possible link between Ang-2 and tumor-associated angiogenesis could be inferred from the histopathologic features of the tumor vessels observed. Tumor vessels appeared to be immature, with tortuous morphology and a relatively small luminal size, significantly different from the ordinary straight vessels in normal liver tissue. Other studies also suggested that Ang-2 may be associated with vessel immaturity. The characteristically small luminal size of tumor vessels was reported in *ANG2* transgenic mice and in Ang-2-dependent corneal neovascularization in mice.[10,13] It is noteworthy that PESCs were not sufficiently recruited to surround ECs in these Ang-2-associated *in vivo* models. In addition, it was demonstrated that overexpression of the *ANG2* gene produced a lower degree of vessel maturation in *in vivo* experiments involving gastric cancer cells.[16] We consistently found that insufficient recruitment of PESCs around ECs became more evident going from normal liver tissue to the tumor periphery, and also going from the periphery to the intermediate portion of the tumor, and that expression of Ang-2, but not Ang-1, increased accordingly with increasing proximity to the center of the tumor (Figs. 6, 10D). Because Ang-1 maintains and stabilizes mature vessels, these findings suggest that high expression of *ANG2* RNA relative to *ANG1* RNA may prevent vessel maturation.

In conclusion, we have demonstrated that Ang-2/*ANG2* is preferentially expressed at the protein and RNA levels in metastatic CRC in the liver. The current data suggest that Ang-2 may cooperate with VEGF in tumor-associated angiogenesis and thus assist in tumorigenesis of CRC metastases in the liver. Therefore, with respect to anti-VEGF therapy, inhibition of Ang-2 activity may be an alternative or additional strategy in the prevention of CRC-related liver metastasis.

Acknowledgment: The authors thank Y. Naito and S. Yamane for their work on the preparation of paraffin sections and A. Ueda, M. Yamada, and X. Xu for their experimental assistance.

References

1. Nelson H, Petrelli N, Carlin A, Couture J, Fleshman J, Guillem J, Miedema B, et al. Guidelines 2000 for colon and rectal cancer surgery. J Natl Cancer Inst 2001;93:583–596.
2. August DA, Ottow RT, Sugarbaker PH. Clinical perspective of human colorectal cancer metastasis. Cancer Metastasis Rev 1984;3:303–324.
3. Fong Y, Cohen AM, Fortner JG, Enker WE, Turnbull AD, Coit DG, Marrero AM, et al. Liver resection for colorectal metastases. J Clin Oncol 1997;15:938–946.
4. Kohn EC, Liotta LA. Invasion and metastasis: new approaches to an old problem. Oncology 1993;7:47–52.
5. Terayama N, Terada T, Nakanuma Y. A morphometric and immunohistochemical study on angiogenesis of human metastatic carcinomas of the liver. HEPATOLOGY 1996;24:816–819.
6. Folkman J. Tumor angiogenesis: therapeutic implications. N Engl J Med 1971;285:1182–1186.
7. Folkman J. What is the evidence that tumors are angiogenesis dependent? J Natl Cancer Inst 1990;82:4–6.
8. Davis S, Aldrich TH, Jones PF, Acheson A, Compton DL, Jain V, Ryan TE, et al. Isolation of angiopoietin-1, a ligand for the TIE2 receptor, by secretion-trap expression cloning. Cell 1996;87:1161–1169.
9. Suri C, Jones PF, Patan S, Bartunkova S, Maisonpierre PC, Davis S, Sato TN, et al. Requisite role of angiopoietin-1, a ligand for the TIE2 receptor, during embryonic angiogenesis. Cell 1996;87:1171–1180.
10. Maisonpierre PC, Suri C, Jones PF, Bartunkova S, Wiegand SJ, Radziejewski C, Compton D, et al. Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis. Science 1997;277:55–60.
11. Wong AL, Haroon ZA, Werner S, Dewhirst MW, Greenberg CS, Peters KG. Tie2 expression and phosphorylation in angiogenic and quiescent adult tissues. Circ Res 1997;81:567–574.
12. Holash J, Wiegand SJ, Yancopoulos GD. New model of tumor angiogenesis: dynamic balance between vessel regression and growth mediated by angiopoietins and VEGF. Oncogene 1999;18:5356–5362.
13. Asahara T, Chen D, Takahashi T, Fujikawa K, Kearney M, Magner M, Yancopoulos GD, et al. Tie2 receptor ligands, angiopoietin-1 and angiopoietin-2, modulate VEGF-induced postnatal neovascularization. Circ Res 1998;83:233–240.
14. Ahmad SA, Liu W, Jung YD, Fan F, Reinmuth N, Bucana CD, Ellis LM. Differential expression of angiopoietin-1 and angiopoietin-2 in colon carcinoma. A possible mechanism for the initiation of angiogenesis. Cancer 2001;92:1138–1143.
15. Tanaka S, Mori M, Sakamoto Y, Makuuchi M, Sugimachi K, Wands JR. Biologic significance of angiopoietin-2 expression in human hepatocellular carcinoma. J Clin Invest 1999;103:341–345.
16. Etoh T, Inoue H, Tanaka S, Barnard GF, Kitano S, Mori M. Angiopoietin-2 is related to tumor angiogenesis in gastric carcinoma: possible in vivo regulation via induction of proteases. Cancer Res 2001;61:2145–2153.
17. Wong MP, Chan SY, Fu KH, Leung SY, Cheung N, Yuen ST, Chung LP. The angiopoietins, tie2 and vascular endothelial growth factor are differentially expressed in the transformation of normal lung to non-small cell lung carcinomas. Lung Cancer 2000;29:11–22.
18. Bunone G, Vigneri P, Mariani L, Buto S, Collini P, Pilotti S, Pierotti MA, et al. Expression of angiogenesis stimulators and inhibitors in human thyroid tumors and correlation with clinical pathological features. Am J Pathol 1999;155:1967–1976.
19. Stratmann A, Risau W, Plate KH. Cell type-specific expression of angiopoietin-1 and angiopoietin-2 suggests a role in glioblastoma angiogenesis. Am J Pathol 1998;153:1459–1466.
20. Holash J, Maisonpierre PC, Compton D, Boland P, Alexander CR, Zagzag D, Yancopoulos GD, et al. Vessel cooption, regression, and growth in tumors mediated by angiopoietins and VEGF. Science 1999;284:1994–1998.
21. Koga K, Todaka T, Morioka M, Hamada J, Kai Y, Yano S, Okamura A, et al. Expression of angiopoietin-2 in human glioma cells and its role for angiogenesis. Cancer Res 2001;61:6248–6254.

22. Ahmad SA, Liu W, Jung YD, Fan F, Wilson M, Reinmuth N, Shaheen RM, et al. The effects of angiopoietin-1 and -2 on tumor growth and angiogenesis in human colon cancer. Cancer Res 2001;61:1255–1259.
23. Muramatsu Y, Takayasu K, Moriyama N, Shima Y, Goto H, Ushio K, Yamada T, et al. Peripheral low-density area of hepatic tumors: CT-pathologic correlation. Radiology 1986;160:49–52.
24. Murakami T, Kim T, Takamura M, Hori M, Takahashi S, Federle MP, Tsuda K, et al. Hypervascular hepatocellular carcinoma: detection with double arterial phase multi-detector row helical CT. Radiology 2001;218: 763–767.
25. Takemasa I, Yamamoto H, Sekimoto M, Ohue M, Noura S, Miyake Y, Matsumoto T, et al. Overexpression of CDC25B phosphatase as a novel marker of poor prognosis of human colorectal carcinoma. Cancer Res 2000;60:3043–3050.
26. Noura S, Yamamoto H, Ohnishi T, Masuda N, Matsumoto T, Takayama O, Fukunaga H, et al. Comparative detection of lymph node micrometastases of Stage II colorectal cancer by reverse transcriptase polymerase chain reaction and immunohistochemistry. J Clin Oncol 2002;20:4232–4241.
27. Hayashi N, Yamamoto H, Hiraoka N, Dono K, Ito Y, Okami J, Kondo M, et al. Differential expression of cyclooxygenase-2 (COX-2) in human bile duct epithelial cells and bile duct neoplasm. HEPATOLOGY 2001;34:638–650.
28. Zhang EG, Smith SK, Baker PN, Charnock-Jones DS. The regulation and localization of angiopoietin-1, -2, and their receptor Tie2 in normal and pathologic human placentae. Mol Med 2001;7:624–635.
29. Willam C, Koehne P, Jurgensen JS, Grafe M, Wagner KD, Bachmann S, Frei U, Eckardt KU. Tie2 receptor expression is stimulated by hypoxia and proinflammatory cytokines in human endothelial cells. Circ Res 2000;87: 370–377.
30. Takahashi Y, Kitadai Y, Bucana CD, Cleary KR, Ellis LM. Expression of vascular endothelial growth factor and its receptor, KDR, correlates with vascularity, metastasis, and proliferation of human colon cancer. Cancer Res 1995;55:3964–3968.
31. Warren RS, Yuan H, Matli MR, Gillett NA, Ferrara N. Regulation by vascular endothelial growth factor of human colon cancer tumorigenesis in a mouse model of experimental liver metastasis. J Clin Invest 1995;95: 1789–1797.
32. Ishigami SI, Arii S, Furutani M, Niwano M, Harada T, Mizumoto M, Mori A, et al. Predictive value of vascular endothelial growth factor (VEGF) in metastasis and prognosis of human colorectal cancer. Br J Cancer 1998;78:1379–1384.
33. Laugmuir VK, Cobleigh MA, Herbst RS, Holmgren E, Hurwitz H, Kabbinavar F, Mille K, et al. Successful long-term therapy with bevacizumab (Azastin) in solid tumors [abstract]. Proc Am Soc Clin Oncol 2002;21:9A.
34. Poon RT, Lau CP, Cheung ST, Yu WC, Fan ST. Quantitative correlation of serum levels and tumor expression of vascular endothelial growth factor in patients with hepatocellular carcinoma. Cancer Res 2003;63:3121–3126.
35. Mitsuhashi N, Shimizu H, Ohtsuka M, Wakabayashi Y, Ito H, Kimura F, Yoshidome H, et al. Angiopoietins and Tie-2 expression in angiogenesis and proliferation of human hepatocellular carcinoma. HEPATOLOGY 2003; 37:1105–1113.
36. Shahrara S, Volin MV, Connors MA. Haines GK, Koch AE. Differential expression of the angiogenic Tie receptor family in arthritic and normal synovial tissue. Arthritis Res 2002;4:201–208.
37. Shweiki D, Itin A, Soffer D, Keshet E. Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. Nature 1992;359:843–845.
38. Clavo AC, Brown RS, Wahl RL. Fluorodeoxyglucose uptake in human cancer cell lines is increased by hypoxia. J Nucl Med 1995;36:1625–1632.
39. Mandriota SJ, Pepper MS. Regulation of angiopoietin-2 mRNA levels in bovine microvascular endothelial cells by cytokines and hypoxia. Circ Res 1998;83:852–859.
40. Oh H, Takagi H, Suzuma K, Otani A, Matsumura M, Honda Y. Hypoxia and vascular endothelial growth factor selectively up-regulate angiopoietin-2 in bovine microvascular endothelial cells. J Biol Chem 1999;274: 15732–15739.
41. Yuan HT, Yang SP, Woolf AS. Hypoxia up-regulates angiopoietin-2, a Tie-2 ligand, in mouse mesangial cells. Kidney Int 2000;58:1912–1919.
42. Lunevicius R, Nakanishi H, Ito S, Kozaki K, Kato T, Tatematsu M, Yasui K. Clinicopathological significance of fibrotic capsule formation around liver metastasis from colorectal cancer. J Cancer Res Clin Oncol 2001;127: 193–199.

外科治療　Vol. 90　No. 1（2004：1）

N0大腸癌における免疫染色による微小転移検出の利点と欠点

山本　浩文　能浦　真吾　高山　治
小川　稔　安井　昌義　万井　真理子
畑　泰司　池永　雅一　池田　正孝
大植　雅之　関本　貢嗣　門田　守人

永井書店

臨床

N0大腸癌における免疫染色による微小転移検出の利点と欠点

Benefit and drawback of immunohistochemical detection of micrometastasis in N0 colorectal cancer

山本浩文 YAMAMOTO Hirofumi
能浦真吾 NOURA Shingo
高山治 TAKAYAMA Osamu
小川稔 OGAWA Minoru
安井昌義 YASUI Masayoshi
万井真理子 MANI Mariko
畑泰司 HATA Taishi
池永雅一 IKENAGA Masakazu
池田正孝 IKEDA Masataka
大植雅之 OHUE Masayuki
関本貢嗣* SEKIMOTO Mitsugu
門田守人** MONDEN Morito

はじめに

微小転移とは，通常の病理検査では検出されない程度のわずかな癌細胞の転移である[1]．免疫染色による微小癌細胞の検出は古くからなされてきており，大腸癌リンパ節中の微小癌細胞の存在と患者予後との関係については盛んに検討されてきたが，その結論はcontroversialである(表1)[2)-11)]．このことは，検索切片数の問題や，どういう場合に微小転移陽性とするかなど診断基準が統一されていないこと，さらには，切片の切削レベルによる再現性の問題などが関係している可能性が考えられる．本研究では，まず検索枚数の違いによる微小転移の検出率について検討し，その結果に基づいて，大腸癌のリンパ節中の微小癌細胞の存在

表1　N0大腸癌の微小転移と予後について

著者	対象	抗体	微小転移	予後
Nicholson 2)	Dukes A, B 33症例，542リンパ節	CAM5.2	 6リンパ節(1.1%)	—
Sasaki 3)	Dukes A, B 19症例，358リンパ節	CAM5.2	19症例(100%) 90リンパ節(25.1%)	—
Yasuda 4)	Dukes B 42症例，1013リンパ節	CAM5.2	32症例(76.2%) 136リンパ節(13.4%)	—
Cutait 5)	Dukes A, B 46症例，603リンパ節	CK(AE1+AE3) CEA	12症例(26%) 22リンパ節(3.7%)	有意差なし
Jeffers 6)	Dukes B 77症例，559リンパ節	CK(AE1+AE3)	19症例(25%)	有意差なし
Adell 7)	Dukes B 100症例，467リンパ節	Anti-CK	39症例(39%) 81リンパ節(17.3%)	有意差なし
Oberg 8)	Dukes A, B 147症例，609リンパ節	CAM5.2	47症例(32%) 77リンパ節(11.6%)	有意差なし
Greenson 9)	Dukes B 50症例，568リンパ節	CK(AE1+AE3)	14症例(28%) 33リンパ節(5.8%)	予後不良
Isaka 10)	Dukes B（直腸癌のみ） 42症例，644リンパ節	CAM5.2	9症例(21.4%) 19リンパ節(2.9%)	予後不良

大阪大学大学院医学系研究科病態制御外科学教室　*講師　**教授
Key words : 大腸癌／微小転移／免疫染色

表2 対象症例

性差	男性	36例
	女性	19例
腫瘍部位	結腸	30例
	直腸	25例
腫瘍径	0.8～12.0cm (5.0±2.4cm)	
年齢	41～80歳 (59.8±8.6歳)	
腫瘍分化度	高分化	31例
	中分化	24例
stage(TNM分類)	stage I	9例
	stage II	46例

様式や癌細胞数，所属リンパ節中の広がり，などこれまで明らかにされていない点について調べ，免疫染色による微小転移診断の臨床的意義とその限界について考察した．

I. 対象と方法

1989～1996年までに当科で治癒切除を受けたN0大腸癌55例を対象とした(表2)．1症例あたりの平均検索リンパ節個数は，12.0個であり，平均術後経過観察期間は80.5±39.0カ月である．術前化学療法や放射線治療は行っていない．術後化学療法は，stage Iの11.1%，stage IIの37.0%に対して，5-FU系薬剤(ときにマイトマイシンCを併用)が投与されていた．ホルマリン固定パラフィン包埋されたのべ662個のリンパ節より，6枚の連続切片を作製し，1枚はHE染色を，5枚はサイトケラチンの免疫染色を行った．また主病巣についても，2枚の切片を作製し，HE染色とサイトケラチン染色を行った．脱パラ後，切片の抗原賦活を行い(クエン酸緩衝液(pH 6.0，10 mM)に95℃40分間温浴)，抗サイトケラチンモノクローナル抗体(AE1/AE3：1μg/ml)とペルオキシダーゼ標識dextran polylinker付加二次抗体(Envision plus(DAKO))を用いて水平式自動免染機による染色を行った．陽性コントロールとして大腸癌組織サンプル，陰性コントロールとして一次抗体の代わりに非免疫マウスIgGを使用した．

II. 結　　果

1．サイトケラチン抗体による大腸癌組織とリンパ節染色

原発巣の検討では，大腸癌組織55例全例でサイトケラチンの発現がみられた(図1A)．正常の細胞成分の中では，リンパ節の骨格を作る紡錘形の細網細胞(reticular cell)がしばしば弱い染色性を

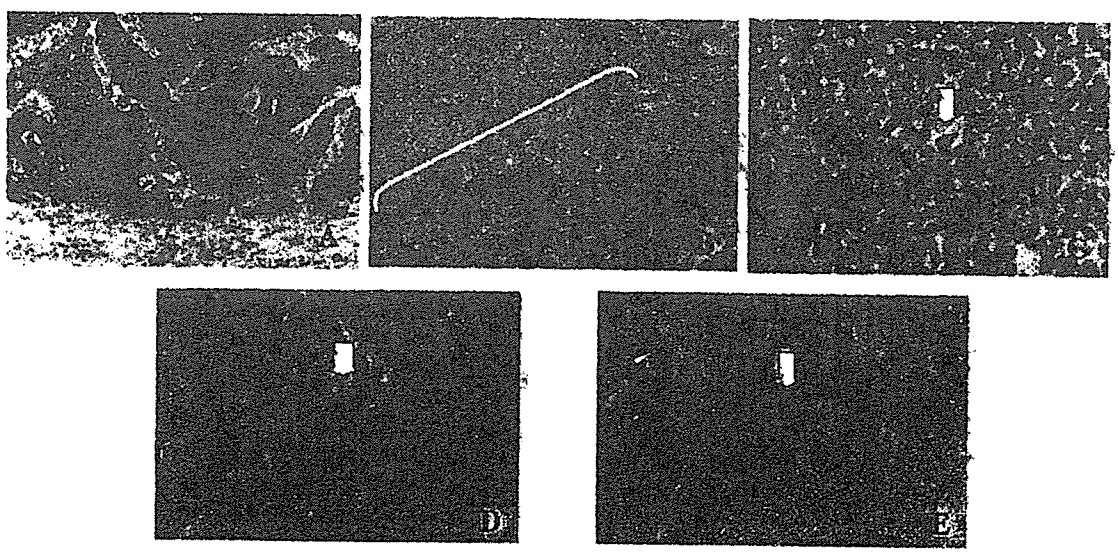

図1 主腫瘍とリンパ節のサイトケラチン染色
A 大腸癌組織のサイトケラチン発現　B 細網細胞　C 組織球　D 癌細胞
E 隣接切片では，癌細胞の一端が切れているので，どの種の細胞か不明

表3　検索切片数による微小転移の検出率

検索切片数	リンパ節	症例数
1切片	4.1%(27/662)	32.7%(18/55)
2切片	5.7%(38/662)	41.8%(23/55)
5切片	11.9%(79/662)	49.1%(27/55)

表4　微小転移の解剖学的広がり

	微小転移陽性数			
	1群	2群	3群	計
リンパ節	59/373	16/203	4/86	79/662(11.9%)
症例	15	8	5	27/ 55(49.1%)

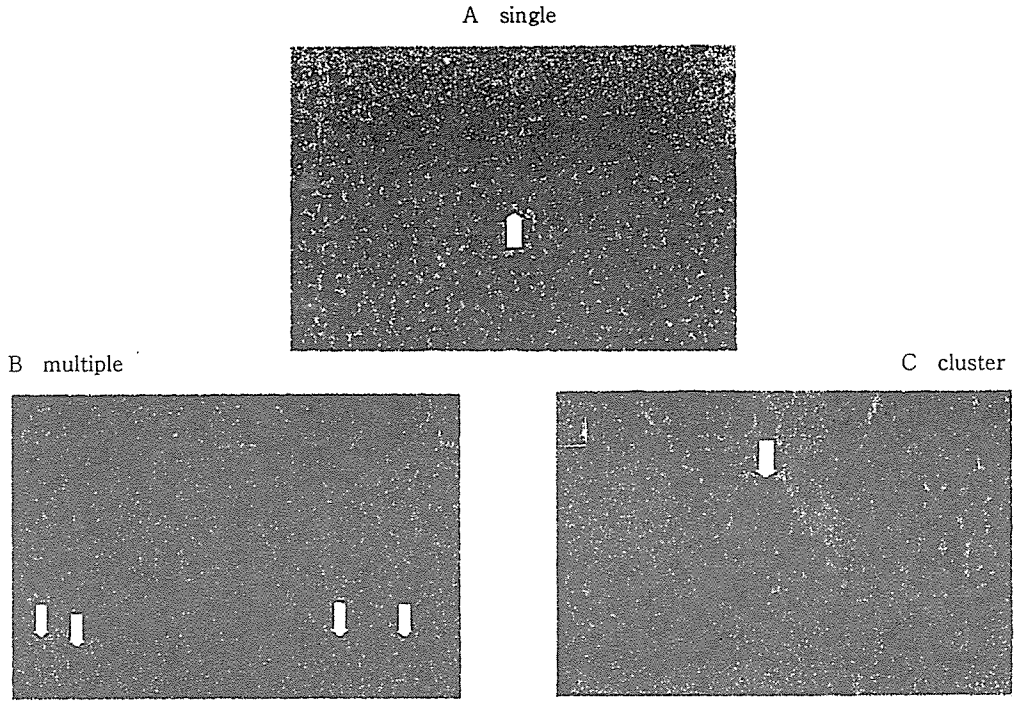

図2　微小転移細胞の存在様式
微小転移細胞の存在様式として，A　微小転移の多くは孤立性であり(single)，B　複数の孤立性細胞が見つかる場合(multiple)，C　さらに島状に集合体を形成するもの(cluster)がある．

示す他に，大食細胞(マクロファージ)もときに染色性を示した(図1B，C)．癌細胞は，形態的に正常細胞との判別が容易である(図1D)．しかし，切片が癌の中心付近ではなく，端をかすめるような場合は，診断が困難であった(図1E)．微小転移の診断にあたっては，5枚の染色切片を二人で検鏡し，サイトケラチンが陽性で，形態的に大きな細胞体と核異型を有し明らかに癌細胞といえるものだけを陽性とした．表3に，検索切片枚数と微小転移検出率の結果について示す．

2．微小転移リンパ節の頻度と分布

微小転移は，N0症例55例中27例(49.1%)に，662個のリンパ節中79個(11.9%)に認められた．微小転移リンパ節の解剖学的広がりを表4に示す．

3．微小転移細胞の存在様式

微小癌細胞は多くは被膜下の類洞かリンパ濾胞周囲の類洞に存在した．その多くは1個の癌細胞としてみつかるが(single)，ときにそのような孤立性細胞が，複数みつかることがあり(multiple)，さらに島状に集合体を形成するものもみられる(cluster)(図2)．微小転移存在様式と微小転移細胞の個数について症例の内訳を表5，6に示す．

表5 微小転移存在様式

存在様式	なし	single cell	culster	single＋culster
症例数	28	22	1	4

表6 微小転移細胞個数

細胞個数	0	1-5	6-10	11-20	20<
症例数	28	16	7	2	2

表7 微小転移と臨床病理学的所見

	リンパ節微小転移		
	陽性 (N＝27)	陰性 (N＝28)	p値
年齢	60.6±8.5	59.0±8.7	$p=0.517$
性			
男性	17	19	$p=0.703$
女性	10	9	
腫瘍占居部位			
結腸	14	16	$p=0.694$
直腸	13	12	
組織型			
高分化	14	17	$p=0.508$
中分化	13	11	
深達度			
～固有筋層	1	8	$p=0.013^*$
漿膜下層～	26	20	
リンパ管侵襲			
陰性	15	17	$p=0.698$
陽性	12	11	
静脈侵襲			
陰性	20	25	$p=0.144$
陽性	7	3	
腫瘍径(cm)	5.7±1.7	4.3±2.8	$p=0.037^*$

＊統計学的有意差有り

4．微小転移と臨床病理学的所見との関係

微小転移と臨床病理学的所見について表7に示す．主腫瘍の深達度が筋層以内に留まっている9例中微小転移陽性はわずか1例のみ(11.1%)であったのに対し，筋層をこえる46例では，26例(56.5%)と高率に微小転移を認めた．また微小転移は腫瘍径とも関連していた．

5．予後因子としての微小転移

臨床病理因子の5年生存率への影響を調べると，分化度，静脈侵襲のみが予後因子となる傾向がみられたが，微小転移の有無とは関連性を認めなかった(表8)．これは，stage I 症例を除いてstage II 症例だけで検討しても同様であった．

次に微小転移の詳細と予後の関係について検討した．すなわち，微小転移を有するリンパ節の①数，②主腫瘍からの距離，③微小癌細胞の数，および④その様式(なし，single cell, cluster 形成)についてである．このなかで唯一，存在様式に着目した解析で術後再発との関連性が示唆された．N0症例55例中，15例(27.3%)に術後5年以内の再発がみられ，cluster を形成していた5例中3例で再発がみられたのに対し，single cell パターンでは22例中5例，微小転移なし群では28例中7

表8 各因子の5生率への影響

臨床病理学的因子	p値
年齢（<60：≧60）	0.919
性（男性：女性）	0.301
腫瘍占居部位（結腸：直腸）	0.664
深達度（～固有筋層：漿膜下層～）	0.831
腫瘍分化度（高分化：中分化）	0.050
リンパ管侵襲（陰性：陽性）	0.156
静脈侵襲（陰性：陽性）	0.083
腫瘍径（<5.0cm：≧5.0cm）	0.532
術後補助化学療法（なし：あり）	0.557
微小リンパ節転移（陰性：陽性）	0.817

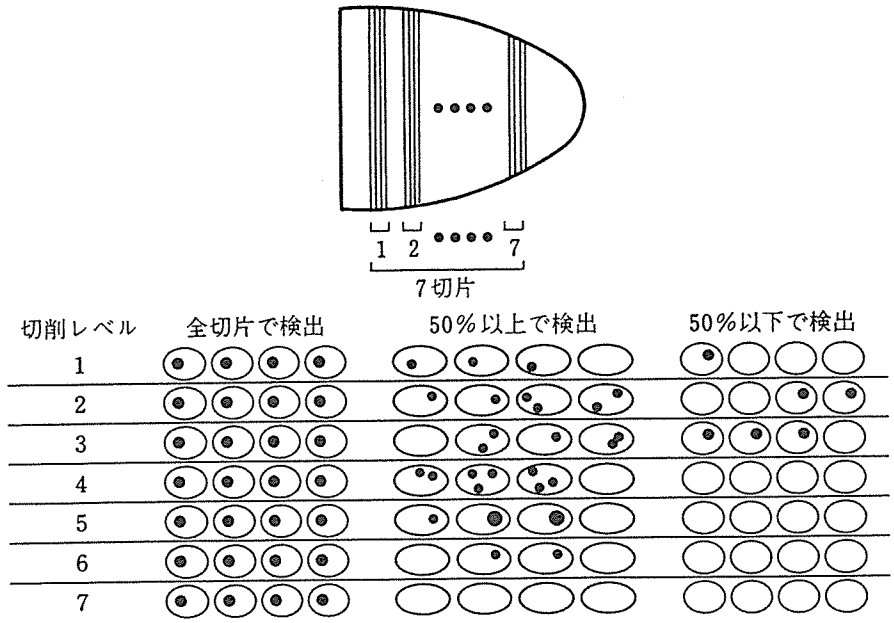

図3　多数切片作製による再現性の検討

例と低率であった.

6．多数切片作製による再現性の検討

微小転移陽性とされた10個のリンパ節について，さらに連続切片4枚を異なる7レベルで作製し計28枚について，微小転移の分布を検討した(図3)．その結果，4個のリンパ節は28切片全てで微小転移が検出されたものの，2個のリンパ節は50%以上の切片で，残り4個のリンパ節では50%以下の切片でのみに微小転移が検出されたにすぎなかった.

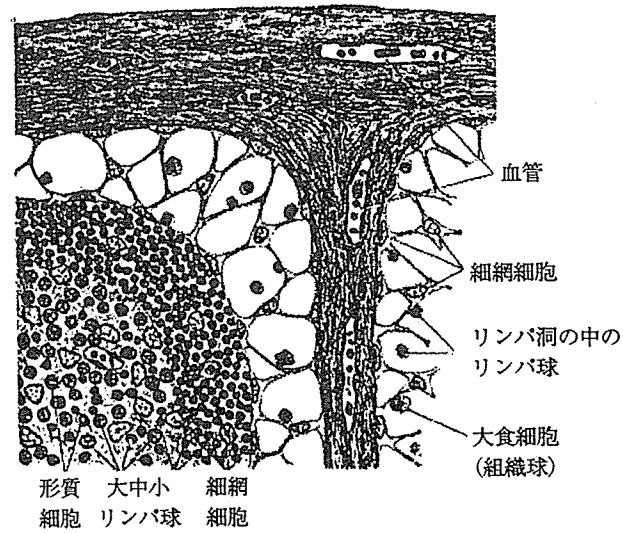

図4　正常リンパ節の構造

III. 考　　察

微小癌細胞の検出にあたっては癌細胞で強発現しており，リンパ節の正常な成分では発現がないか，あっても僅かな分子が適当である．これまでの免疫染色を用いた報告の多くはサイトケラチンがマーカーとして利用され，一部でCEAが用いられている．サイトケラチンの検出にはAE1/AE3抗体(DAKO社)が頻用されており，CAM5.2(BectonDickinson社)がときに使用されている．リンパ節を構成する細胞として，リンパ球の他に，細網細胞，大食細胞，形質細胞などがある(図4)[12].

われわれの検討では，細網細胞が，しばしば弱い染色性を示したが，紡錘形の特徴ある形態から癌細胞と誤認することはない．ときに大食細胞や組織球が染まることがあるが，やはり癌細胞との

識別は容易である．本研究の結果には示していないがpreliminaryにCEA染色も行った．CEAはマクロファージで発現がみられる他，微細構造物が類洞を流れるパターンがしばしばみられた．これは，分泌型のCEA蛋白が類洞内を流れてきたものを捉えているものと考えられる．原発巣では，CEAは腺管形成の内面を中心とする強い染色性がみられたが，微小癌細胞の染色性についてはCEAよりもサイトケラチンの方がむしろ強かったので，本研究ではサイトケラチン抗体(AE1/AE3)を利用することとした．

これまでのほとんどの報告では，微小転移は1枚の切片で診断されている．これは微小転移細胞が広くリンパ節全体に広がっているのではないかという楽観的な見解に基づいているのと，多数のリンパ節を検索するのに複数の切片を調べることは膨大な仕事量となるからである．われわれは，あえて662個のリンパ節について，連続6切片を作製し徹底的に微小転移細胞を探索した．微小転移陽性のリンパ節の頻度は1枚，2枚，5枚と切片数を増やすにつれ，明らかに増加した．その主要因として，1枚では癌の確定診断が困難なことが多いが，両隣の隣接切片を染色してみて初めて大きな核，明瞭な核小体がはっきりと描出され癌細胞と判別できることがあげられる．癌細胞の中央で切片が切られている場合は問題ないが，細胞の端の方をかすめていて僅かに染色性がみとめられる場合は，どのような細胞が染まっているのか形態的に判断できないことをしばしば経験した(図1E)．また，微小転移診断にあたっては，検鏡を2人で行い，サイトケラチンが陽性で，形態的に明らかに癌細胞といえるものだけを陽性とした．このような判定基準の明確化は，検査に普遍性をもたらすうえで重要である．

N0大腸癌の微小転移は実に約半数(49.1%)の例で認められ，この数字は当初のわれわれの想像をはるかに超えるものであった．表4に微小転移リンパ節を解剖学的位置に照らし合わせてみると，1群リンパ節の15.8%，2群，3群リンパ節の7.9%，4.7%と，遠位リンパ節にも少なからずの微小転移が存在した．本邦ではN0でもある程度の予防的リンパ節郭清が行われているが，この結果は多くの微小転移がこれにより除去されていることを示している．

微小転移の有無が，腫瘍の大きさ，ことに深達度と深く関連していたことは，特筆すべきことである．癌が，粘膜下層・筋層に留まるとリンパ節に微小癌細胞が検出される率は10%程度であるが，筋層を越えると微小転移の率は激増する．このことは，免疫染色による微小転移検出が大腸癌の初期進展を的確に表していることを物語っている．本来，免疫染色はたったひとつの癌細胞でも検出する超高感度検査法であり，多数の切片を調べる限りは，きわめて微小な癌細胞をみつけるのに強力な効果を発揮する．例えばセンチネルリンパ節中の微小転移の検索にはRT-PCR法よりもむしろ多数切片検索による免疫染色が有用であると筆者は考えている．

免疫染色で検出される微小転移は予後因子になりえないという多くの報告がある(表1)．一方，GreensonやIsakaらは予後因子となりうるとしている[9)10)]．このような意見の相違は，検討症例数や研究デザインの違いなどが関係していると考えられるが，それに加えて，今回の多切片解析の結果は，切片の選び方によって陽性，陰性結果が大きく変わりうることを示しており，このような再現性の不安定性が，これまでのcontroversialな状況と関連しているのかもしれない．

もうひとつ考えられるのは，免疫染色では高感度ゆえに，single cellレベルの微小転移を数多く捉えてしまうことが，予後を予測するうえで問題となるのではないかということである．過去のほとんどの報告は，1枚の検索のみであり，その検出率はN0症例として21.4～39.0%である[5)-10)]．今回われわれは5枚の検索によって，約50%に微小転移を認め，Yasuda[4)]らは，やはり5枚の検索で76.2%，Sasaki[3)]らは10枚法で100%と報告している．

このように，single cellレベルまで含めると微小転移は非常に高頻度に存在するので，その予後

因子としての意義は，単に存在するか否かではなく，むしろその量が多いか少ないか，あるいは集合体を形成していくかどうかという点がより重要である可能性がある．今回の検討でも，single cell を有する症例に比べて，cluster を形成している症例で高率に再発がみられた．この点についてわれわれは，微小転移といえども n(+)に匹敵する程の癌細胞が存在するものがあることを定量的 RT-PCR 法で確認している[11]．さらに RT-PCR 法で少数ながら prospective に検討すると，微小転移が予後予測に有用であった例を経験した[13]．他に，Liefers らは，同様の結果を報告している[14]．RT-PCR の最大の利点は，リンパ節全体をすりつぶして検索できることであり，免疫染色で問題となった切片のレベルによる結果の再現性についての問題がないことがあげられる．

おわりに

5 枚連続切片を用いた免疫染色は，微小転移診断に確実性を与え有用であった．また single cell を含めると，N0大腸癌の約半数でリンパ節微小転移がみつかった．免疫染色による微小転移は主腫瘍の深達度や腫瘍径とよく相関し，癌の初期進展，すなわち local disease(腸管の限局病変)から expanding disease(周辺のリンパ節に拡がりつつある病変)への移行を的確に反映する．しかし，免疫染色で捉えられる微小転移は cluster 形成例で再発への関連性が示唆されたが，全体としては術後再発の予測因子とはならず，single cell 程度のリンパ節転移は再発の予測因子とならないと考える．

文　献

1) Wells CA, Heryet A, Brochier J, et al: The immunocytochemical detectyion of axillary micrometastasis in breast cancer. Br J Cancer 50: 193-197, 1984.
2) Nicholson AG, Marks CG, Cook MG: Effect on lymph node status of triple levelling and immunohistochemistry with CAM5.2 on node negative colorectal carcinomas. Gut 35: 1447-1448, 1994.
3) Sasaki M, Watanabe H, Jass JR, et al: Occult lymph node metastases detected by cytokeratin immunohistochemistry predict recurrence in "node-negative" colorectal cancer. J Gastroenterol 32: 758-764, 1997.
4) Yasuda K, Adachi Y, Shiraishi N, et al: Pattern of lymph node micrometastases and prognosis of patients with colorectal cancer. Ann Surg Oncol 8: 300-304, 2001.
5) Cutait R, Alves VAF, Lopes LC, et al: Restaging of colorectal cancer based on the identification of lymph node micrometastases through immunoperoxidase staining of CEA and cytokeratins. Dis Colon Rectum 34: 917-920, 1991.
6) Jeffers MD, O'Dowd GM, Mulcahy H, et al: The prognostic significance of immunohistochemically detected lymph node micrometastases in colorectal carcinoma. J Pathol 172: 183-187, 1994.
7) Adell G, Boeryd B, Franlund R, et al: Occurrence and prognostic importance of micrometastases in regional lymph nodes in Dukes' B colorectal carcinoma: An immunohistochemical study. Eur J. Surg 162: 637-642, 1996.
8) Oberg A, Stenling R, Tavelin B,et al: Are lymph node micrometastases of any clinical significance in Dukes stage A and B colorectal cancer? Dis Colon Rectum 41: 1244-1249, 1998.
9) Greenson JK, Isenhart CE, Rice R, et al: Identification of occult micrometastases in pericolic lymph nodes of Dukes' B colorectal cancer patients using monoclonal antibodies against cytokeratin and CC49. Correlation with long-term survival. Cancer 73: 563-569, 1994.
10) Isaka N, Nozue M, Doy M, et al: Prognostic significance of perirectal lymph node micrometastases in Dukes' B rectal carcinoma: An immunohistochemical study by CAM5.2. Clin Cancer Res 5: 2065-2068, 1999.
11) Miyake Y, Fujiwara Y, Ohue M, et al: Quantification of micrometastases in lymph nodes of colorectal cancer using real-time fluorescence polymerase chain reaction. Int J Oncol 16: 289-293, 2000.
12) 藤田尚男，藤田恒夫：標準組織学各論．(第 2 版)医学書院，東京，pp39-44，1984.
13) Miyake Y, Yamamoto H, Fujiwara Y, et al: Extensive micrometastases to lymph nodes as a marker for rapid recurrence of colorectal cancer: a study of lymphatic mapping. Clin Cancer Res 7: 1350-1357, 2001.
14) Liefers GJ, Cleton-Jansen AM, van de Velde CJH, et al: Micrometastses and survival in stage II colorectal cancer. N Engl J Med 339: 223-228, 1998.

大腸癌肝転移切除後長期生存例の検討

棚田 稔 久保 義郎 石崎 雅浩
青儀健二郎 栗田 啓 高嶋 成光*

[*Jpn J Cancer Chemother* 31(11): 1659-1661, October, 2004]

The Long-Term Results of Hepatic Resection for Metastatic Lesions from Colorectal Cancer: Minoru Tanada, Yoshiro Kubo, Masahiro Ishizaki, Kengiro Aogi, Akira Kurita and Shigemitsu Takashima (*Dept. of Surgery, National Shikoku Cancer Center Hospital*)

Summary

We reviewed the clinical course of 51 patients who underwent hepatic resection for metastatic lesions from colorectal cancer between January 1984 and December 1997. The cumulative survival rate at 3 and 5 years were 57% and 43%, respectively. Sex, age, chronology of liver metastases (LM), number of LM, maximum diameter of LM, macroscopic surgical resection margin, type of hepatic resection, chemotherapy after hepatic resection, and site of primary tumor were not found to be statistically significant prognostic factors. The presence of lymph node metastases for the primary tumor was a predictor of shorter survival duration by univariate analysis ($p=0.03$).

Recurrence was not observed in 15 patients. However, recurrence was observed in 36 patients, of which 4 were in remission by undergoing repeated resection for recurrence sites (2 were in lung, 2 were in liver). Although the long term survival of the 19 patients with no significant remarks to be noted, but no one survived with more than 4 hepatic metastases among the long term survivors. Key words: Colorectal cancer, Liver metastases, Hepatic resection

要旨 1984年1月より1997年12月までに51例の大腸癌肝転移に対し肝切除を行った。51例の3，5年生存率はそれぞれ57，43%であった。予後因子の検討では，性別，年齢，再発時期，肝再発腫瘍径，肝再発個数，肝切除術式，肝切除断端，肝切除後の補助化学療法の有無，原発巣の部位の各因子では予後に差を認めず，原発巣のリンパ節転移陽性例は有意に予後不良であった($p=0.03$)。15例は無再発生存中であり，再発36例中再発巣を切除した4例が無病生存中である。長期生存19例に特徴的所見は認められなかったが，肝転移個数4個以上に長期生存例は認められなかった。

はじめに

大腸癌肝転移に対して積極的に切除が行われ，各施設で良好な成績が報告されている[1-3]。

今回われわれは，当院での大腸癌肝転移切除例の長期フォローアップより，長期生存例について検討した。

I. 対象と方法

1984年1月から1997年12月までに当院外科で切除し，5年以上経過観察できた大腸癌肝転移51例を対象とした。再発例は36例で，36例中4例が再発巣の切除により長期無病生存中である(肝転移2例，肺転移2例)。この4例と無再発生存中の15例の，計19例の臨床病理学的特長について検討した。

累積生存率はKaplan-Meier法にて算出し，logrank testで検定，危険率5%未満($p<0.05$)を有意とした。

II. 結 果

1. 肝転移切除例

大腸癌肝転移切除51例の背景因子は，男性33例，女性18例，平均年齢62歳(40～83歳)，同時性27例，異時性24例，平均腫瘍径3.7 cm (0.5～9.6 cm)，肝転移個数は，1個31例，2個8例，3個6例，4個4例，5個1例，6個1例，切除術式は，部分切除34例，1区域切除7例，2区域切除9例，3区域切除1例，切除断端1 cm未満23例，1 cm以上28例，術後補助化学療法施

連絡先: 〒790-0007 松山市堀之内13 *独立行政法人国立病院機構四国がんセンター・外科
棚田 稔

0385-0684/04/¥500/論文/JCLS

行例は18例で，原発巣は結腸26例，直腸25例，原発巣のリンパ節転移陽性例は34例であった。

2. 肝転移切除後の予後

3，5年生存率はそれぞれ57，43%で，3，5年無再発生存率はそれぞれ31，29%であった(平均観察期間71か月)（図1)。

性別，年齢，転移時期，転移個数，転移腫瘍径，切除断端，切除術式，切除後の補助化学療法，原発巣の部位，原発巣のリンパ節転移の有無の各因子での予後の検討では，原発巣のリンパ節転移陽性例は有意に予後不良であった（p=0.05）（表1)。

3. 再　発

肝切除後の再発は36例に認められ，再発部位は肝22例，肺16例，腹膜2例，原発巣局所1例，骨1例，皮下1例で，再発治療は切除6例，化学療法18例，放射線療法3例，無治療9例であった。

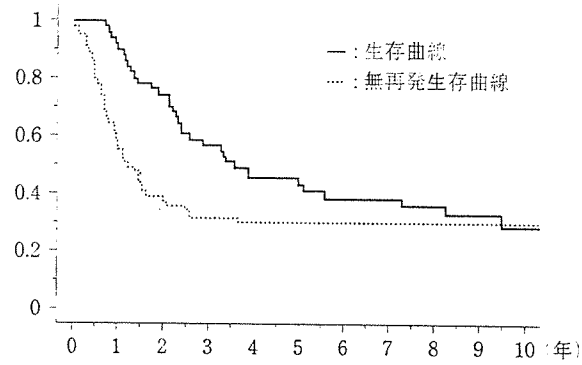

図1　大腸癌肝転移切除例の予後

4. 再発後長期生存例

再発後長期生存例は4例である（表2)。症例1は，肝転移切除後10か月目に肝再発し，再肝切除，初回肝切除後127か月無病生存中，症例2は肝転移切除後44か月目に肺再発し，切除，初回肝切除後112か月無病生存中，症例3は肝転移切除後31か月目，47か月目に肺再発し，それぞれ切除，初回肝切除後70か月無病生存中，症例4は肝転移切除後8か月目に肝再発し，再肝切除，初回肝切除後67か月無病生存中である。

5. 長期生存例

無再発例15例と再発後長期生存例の4例を合わせた19例の背景因子は表のごとくであった（表3)。

III. 考　察

当院における大腸癌肝転移切除51例中5年以上無病生存中の症例は19例であった。これら長期生存例に特徴的な所見は認められなかった。15例は肝転移切除後無再発で生存しているが，再発例でも再発巣が切除により完全にコントロールされている4症例で長期生存例が認められている。長期生存例の平均観察期間は107か月で，肝転移切除後4年以降に再発を認めた症例はなかった。予後因子の検討では，原発巣のリンパ節転移陽性例は有意に予後不良であったが，長期生存例でも19例中9例に原

表1　大腸癌肝転移切除例の予後因子

因　子		症例数	5年生存率（%）	p値
性別	男性：女性	33：18	45：39	0.91
年齢	59≦：60≦	21：30	48：40	0.29
転移時期	同時性：異時性	27：24	37：50	0.08
転移個数	単発：多発	31：20	51：30	0.12
腫瘍径	2 cm＞：2 cm≦	15：36	27：50	0.14
切除断端	1 cm＞：1 cm≦	28：23	39：46	0.76
切除術式	部切：区域切除	34：17	38：53	0.83
補助化学療法	あり：なし	23：28	43：43	0.90
原発巣	結腸：直腸	26：25	39：48	0.32
原発巣のリンパ節転移	(−)：(+)	17：34	64：32	0.03

表2　大腸癌肝転移切除後再発例で長期生存中の症例

症例	年齢・性別（歳）	再発時期	肝転移個数	腫瘍径（cm）	切除術式	切除断端（mm）	補助療法	原発巣	リンパ節転移	再発部位	無病期間
1	58歳・男性	同時性	3	2.7	部分切除	10	−	結腸	+	肝	10か月
2	66歳・男性	異時性	1	0.8	部分切除	5	+	直腸	−	肺	44か月
3	54歳・男性	異時性	1	3.5	1区域切除	8	+	直腸	+	肺	31か月
4	76歳・男性	異時性	1	1.5	部分切除	5	−	直腸	−	肝	8か月

表 3 大腸癌肝転移切除後長期生存例の背景因子

因子		長期生存例 (n=19)	その他 (n=32)
性別	男性：女性	12：7	21：11
年齢（歳）		63（40～78）	62（40～83）
再発時期	同時性：異時性	9：10	28：14
腫瘍径（cm）		3.2（0.5～8.0）	3.4（0.5～9.6）
肝転移個数	1：2：3：4：5：6	14：2：3：0：0：0	17：6：3：4：1：1
切除術式	部分切除	13	21
	1区域切除	3	4
	2区域切除	3	6
	3区域切除	0	1
切除断端	1 cm未満：1 cm以上	10：9	14：18
術後補助化学療法	あり：なし	6：13	12：20
原発部位	結腸：直腸	9：10	17：15
原発巣リンパ節転移	なし：あり	10：9	7：25

発巣のリンパ節転移を認めていた。また、肝転移個数4個以上に長期生存例を認めていない。

結　語

大腸癌肝転移切除後長期生存例に特徴的な所見は認められなかった。再発症例でも、切除で再発巣を完全に摘出できた症例で長期生存を認めた。

文　献

1) 中島　進，鈴木昌八：転移性肝癌に対する治療戦略．日外会誌 **104**(10)：701-706，2003．
2) 香川正己，幕内雅敏：大腸癌 外科治療．日外会誌 **104**(10)：721-729，2003．
3) Kato T, Yasui K, Hirai T, *et al*: Therapeutic results for hepatic metastasis of colorectal cancer with special reference to effectiveness of hepatectomy: Analysis of prognostic factors for 763 cases recorded at 18 institutions. *Dis Colon Rectum* **46**(*10 Suppl*): S22-31, 2003.

Phase II Study of Oral S-1 for Treatment of Metastatic Colorectal Carcinoma

Kuniaki Shirao, M.D., Ph.D.[1]
Atsushi Ohtsu, M.D., Ph.D.[2]
Hideho Takada, M.D., Ph.D.[3]
Yasushi Mitachi, M.D., Ph.D.[4]
Kosei Hirakawa, M.D.[5]
Noboru Horikoshi, M.D.[6]
Takeshi Okamura, M.D., Ph.D.[7]
Koichi Hirata, M.D., Ph.D.[8]
Soh Saitoh, M.D.[9]
Hiroharu Isomoto, M.D.[10]
Atsushi Satoh, M.D., Ph.D.[11]

[1] Department of Gastrointestinal Oncology, National Cancer Center Hospital, Tokyo, Japan.

[2] Department of Gastrointestinal Oncology/Digestive Endoscopy, National Cancer Center Hospital East, Kashiwa, Japan.

[3] Department of Surgery, Kori Hospital, Kansai Medical University, Osaka, Japan.

[4] Department of Gastroenterology, Sendai Koseikai Hospital, Sendai, Japan.

[5] Department of Surgical Oncology, Graduate School of Medicine, Osaka City University, Osaka, Japan.

[6] Department of Medical Oncology, Cancer Institute Hospital, Tokyo, Japan.

[7] Department of Gastroenterologic Surgery, National Kyushu Cancer Center, Fukuoka, Japan.

[8] Department of Surgery I, Sapporo Medical University, Sapporo, Japan.

[9] Department of Gastroenterology, Aomori Prefectural Central Hospital, Aomori, Japan.

[10] Department of Surgery, Kurume University Medical Center, Kurume, Japan.

[11] Department of Gastroenterology, Toyosu Hospital, Showa University, Tokyo, Japan.

Supported by Taiho Pharmaceutical Co. (Tokyo, Japan).

The authors thank Dr. Tetsuo Taguchi, Dr. Hisashi Furue, Dr. Shigeru Tsukagoshi, Dr. Kiyokazu Yoshida, and Dr. Yasuo Ohashi for their kind advice; Dr. Yuh Sakata, Dr. Wasaburo Koizumi, Dr. Yoshihiko Maehara, and Dr. Naoki Hirabayashi for performing extramural review; and all other participants in the S-1 Cooperative Colorectal Carcinoma Study Group. The authors also thank Takeshi Tahara and Koichiro Hoashi for their assistance with data management and Prof. J. Patrick Barron of the International Medical Communications Center at Tokyo Medical University (Tokyo, Japan) for his careful review of the current article.

Address for reprints: Kuniaki Shirao, M.D., Ph.D., National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan; Fax: (011) 81 3 3542 3815; E-mail: kshirao@ncc.go.jp

Received September 24, 2003; revision received February 27, 2004; accepted March 17, 2004.

BACKGROUND. The goal of the current study was to evaluate the objective response rate and toxicity associated with the oral fluoropyrimidine S-1 (a combination of tegafur, 5-chloro-2,4-dihydroxypyridine, and potassium oxonate) in patients with previously untreated metastatic colorectal carcinoma.

METHODS. Thirty-eight patients were enrolled in the study. S-1 was administered orally at a dose of 40 mg/m^2 twice daily for 28 days, followed by a 14-day rest period. Treatment was repeated every 6 weeks unless disease progression was observed.

RESULTS. A combined total of 173 courses of S-1 were administered to the 38 enrolled patients. The median number of courses administered to a given patient was 3.5 (range, 1–18). Although no patient exhibited a complete response to treatment, 15 had partial responses (response rate, 39.5%; 95% confidence interval, 24.0–56.6%). In addition, 5 patients had minor responses, and 14 had stable disease. Four patients were found to have progressive disease after two courses of treatment. The median survival time was 358 days (95% confidence interval, 305–490 days), and the 1-year survival rate was 47.4%. The most common adverse reactions included myelosuppression and gastrointestinal toxicity; most cases involved Grade 1 or 2 toxicity, but Grade 3 toxicities (anemia [7.9% of patients], neutropenia [5.3% of patients], diarrhea [2.6% of patients], and abnormal bilirubin levels [7.9% of patients]) also were noted. Neither Grade 4 toxicity nor treatment-related death was observed during the study.

CONCLUSIONS. Orally administered S-1 is active against metastatic colorectal carcinoma and has an acceptable toxicity profile. This promising agent has the potential to become a valuable chemotherapeutic option. *Cancer* **2004;100: 2355–61.** © *2004 American Cancer Society.*

KEYWORDS: colorectal carcinoma, S-1, 5-fluorouracil derivative, oral fluoropyrimidine, Phase II study.

Colorectal carcinoma is one of the most common causes of malignancy-related death in the United States, Japan, and most European countries. The median survival duration for patients with metastatic colorectal carcinoma treated with supportive care alone is approximately 4–6 months.[1] Systemic chemotherapy with 5-fluorouracil (5-FU) recently was shown to prolong survival, with a median

DOI 10.1002/cncr.20277
Published online 28 April 2004 in Wiley InterScience (www.interscience.wiley.com).

survival time of 17–21 months associated with such treatment.[2,3] The administration of irinotecan together with 5-FU and leucovorin (LV) as first-line treatment for metastatic disease also has been shown to produce a survival benefit,[2,4] but recently, concern has been raised regarding the toxicity of the weekly bolus combination of these agents.[5]

A randomized cooperative group study has yielded preliminary data supporting the role of 5-FU and LV administered via continuous intravenous infusion (CVI) as the backbone of treatment strategies for metastatic colorectal carcinoma.[6] Nonetheless, CVI performed using a portable pump and an indwelling catheter is challenging and may induce phlebitis or infection originating at the injection site and requiring long-term hospitalization; thus, oral anticancer agents have been developed to address this problem.[7] The results of large Phase III studies of oral capecitabine and the combination of tegafur + uracil (UFT) with LV were reported recently and demonstrated survival benefits that were equivalent to those achieved using intravenous 5-FU + LV.[8–11] Oral chemotherapy has major advantages over intravenously administered treatment in terms of pharmacoeconomic considerations and patient preferences, because oral treatment can be administered on an outpatient basis, thereby reducing the length of patients' hospital stays.[12] Over time, the role of oral chemotherapy in the treatment of malignant disease is expected to become increasingly significant.

Gastrointestinal side effects represent the dose-limiting toxicity associated with 5-FU in a long-term administration schedule (i.e., a CVI schedule).[7] Therefore, to maximize the therapeutic effects of 5-FU, prevention of gastrointestinal toxicity is of primary importance. A new oral fluoropyrimidine, S-1, has been developed by Taiho Pharmaceutical Co. (Tokyo, Japan) and adapted for use in the treatment of advanced gastric[13–15] and head and neck malignancies[16]; at present, this agent is used widely throughout Japan. S-1 consists of tegafur, 5-chloro-2,4-dihydroxypyridine (CDHP), and potassium oxonate in a molar ratio of 1:0.4:1.[17] Tegafur is a precursor of 5-FU and functions as an effector. As an enhancer of the antitumor activity of tegafur, CDHP is prescribed to potently and reversibly inhibit the 5-FU degradation enzyme dihydropyrimidine dehydrogenase (DPD); by inhibiting DPD, CDHP induced the long-term retention of an increased concentration of 5-FU in the blood.[18] Orally administered potassium oxonate is selectively distributed to the gastrointestinal tract with high concentration and inhibits orotate phosphoribosyltransferase, which phosphorylates 5-FU to yield the active metabolite form of 5-FU in humans.[19]

In rats bearing subcutaneous Yoshida sarcoma compared with UFT administered at an equally harmful dose to the rats, S-1 tended to maintain the concentration of 5-FU in plasma and tumor tissue for a longer duration and with less gastrointestinal toxicity.[20] Furthermore, compared with tegafur, UFT, and other fluoropyrimidines, S-1 exhibited greater therapeutic efficacy against various rat tumors and human xenografts.[21]

In a Phase I study involving Japanese patients, S-1 was administered orally for 28 days. The maximum allowed dose of S-1 was 150 mg once daily or 75 mg twice daily, and leukopenia was the resulting dose-limiting toxicity. The pharmacokinetic profile of S-1 revealed that twice-daily administration preserved therapeutic 5-FU levels without increasing the maximum 5-FU concentration in the blood.[22,23] Therefore, oral administration of S-1 at a dose of 75 mg twice daily for 28 consecutive days, with a subsequent 14-day rest period, was recommended. Two Phase II studies of twice-daily S-1 administered as a single agent for the treatment of metastatic gastric malignancy yielded response rates of approximately 50%, with minimal toxicity.[13–15]

Based on these results, two Phase II studies of S-1 in the treatment of metastatic colorectal carcinoma were initiated. Response rates of 17% and 35% were observed in these two trials.[13,24] To verify the reproducibility of these findings, we performed our own Phase II study of S-1 in the treatment of Japanese patients with metastatic colorectal carcinoma.

MATERIALS AND METHODS

Eligibility

Patients were entered into the study only if they fulfilled the following eligibility requirements: 1) histologically confirmed colorectal carcinoma; 2) inoperable metastatic disease or recurrent metastatic disease after surgery; 3) the presence of measurable or evaluable lesions; 4) age $\geq$ 20 years but $<$ 75 years; 5) Eastern Cooperative Oncology Group performance status (PS) $\leq$ 2; 6) no previous chemotherapy or radiotherapy for advanced disease (with any adjuvant chemotherapy for colorectal carcinoma required to have been completed $\geq$ 6 months before enrollment); 7) adequate bone marrow function (hemoglobin concentration $\geq$ 9.0 mg/dL, white blood cell count $\geq$ 4000/μL but $\leq$ 12,000/μL, and platelet count $\geq$ 100,000/μL); 8) adequate liver function (serum bilirubin levels $\leq$ 1.5 mg/dL, serum transaminase levels $\leq$ 100 international units per liter, and serum alkaline phosphatase levels $<$ 2 times the upper limit of normal); 9) adequate renal function (serum creatinine levels within normal limits); 10) no other severe med-

ical conditions; and 11) no other active malignancies. In addition, patients were required to provide written informed consent, and pregnant women were excluded from the study.

Treatment Schedule

S-1 was administered at a dose of 40 mg/m^2 twice daily for 28 consecutive days, with a subsequent 14-day rest period. Patients were assigned on the basis of body surface area (BSA) to receive one of the following doses twice daily: 40 mg (BSA < 1.25 m^2), 50 mg (BSA ≤ 125 to < 1.50 m^2), or 60 mg (BSA > 1.50 m^2). S-1 was supplied by Taiho Pharmaceutical Co. in the form of 20 and 25 mg capsules (i.e., 20 and 25 mg tegafur). A course of therapy was defined as 28 consecutive days of treatment followed by a 14-day rest period, and courses were repeated every 6 weeks until either disease progression or unacceptable toxicity was observed. Patients whose toxicities necessitated a rest period of more than 4 weeks were withdrawn from treatment. Prophylactic use of antiemetic agents was not allowed. For all patients, treatment compliance and receipt of treatment without hospitalization were verified by patient interviews conducted on a regular schedule.

Evaluation

Before entry into the study, patients were evaluated using appropriate investigational methods to determine the extent of disease. A complete blood cell count, liver function testing, renal function testing, and urinalysis were performed at least once every 2 weeks during treatment. Appropriate investigation was repeated as necessary to evaluate target lesion sites before every treatment course. Antitumor activity was evaluated in accordance with the general rules, based on the corresponding World Health Organization criteria, set forth by the Japanese Research Society for Colorectal Carcinoma.[25] Complete response (CR) was defined as the disappearance of all evidence of malignant disease for more than 4 weeks. Partial response (PR) was defined as a reduction (lasting longer than 4 weeks) of greater than 50% in the sum over all lesions of the product of the longest perpendicular tumor dimensions, with no evidence of new lesions or of the progression of any preexisting lesion. Stable disease (SD) was defined as a reduction of less than 50% or an increase of less than 25% in the sum over all lesions of the product of the longest perpendicular tumor dimensions, with no evidence of new lesions. Progressive disease (PD) was defined by increases of greater than 25% in sum overall lesions of the product of the longest perpendicular tumor dimensions or the appearance of new lesions. The toxicity criteria of the Japan Society for Cancer Therapy, which were based (with some modification) on the World Health Organization criteria, were used to evaluate treatment-related toxicity.[26] The eligibility and suitability of patients for assessment and the responses of patients to treatment were reviewed extramurally.

TABLE 1
Patient Characteristics

Characteristic	No. of patients
No. of eligible patients	38
Median age in yrs (range)	58.5 (28–74)
Gender (%)	
Male	18 (47)
Female	20 (53)
ECOG PS (%)	
0	18 (47)
1	20 (53)
Primary lesion site (%)	
Colon	23 (61)
Rectum	15 (39)
Histology (%)	
Well/moderately differentiated	33 (87)
Poorly differentiated	5 (13)
Previous therapy (%)	
Surgery	23 (61)
Surgery + adjuvant chemotherapy	4 (11)
Surgery + radiotherapy	2 (5)
None	9 (24)
Mean body surface area in m^2 (range)	1.53 (1.26–1.85)

ECOG PS: Eastern Cooperative Oncology Group Performance Status.

Statistical Methods

Previous Phase II studies have reported a 35.5% response rate for metastatic colorectal carcinoma treated with S-1. The current study was designed to have a target activity level of 35% and a minimum activity level of 15%, with an α error of 0.05 and a β error of 0.2; thus, a minimum of 38 patients were required. Survival was calculated from the date of treatment initiation using the Kaplan–Meier method.

Ethical Considerations

The current trial was approved by the institutional review boards of the clinical oncology programs at all participating hospitals. Approval was based on the 1975 revision of the Helsinki Declaration. Oral and written statements of informed consent were acquired from all patients.

RESULTS

Thirty-eight patients (18 men and 20 women) with advanced metastatic colorectal carcinoma were en-

TABLE 2
Body Surface Area and Corresponding S-1 Dose

BSA (m^2)	S-1 dose[a] (mg)	No. of patients (%)
< 1.25	40	0
≤1.25 to < 1.50	50	15 (39)
≥1.50	60	23 (61)

BSA: body surface area.
[a] Dose administered twice daily

TABLE 3
Objective Response Data

Response type	No. of patients
Complete response	0
Partial response	15
Minor response	5
Stable disease	14
Progressive disease	4
Overall response rate[a]	39.5% (15/38)
95% confidence interval	24.0–56.6%

[a] Includes complete responses and partial responses.

tered into the trial between June 1999 and December 2000. Patient characteristics are summarized in Table 1. The median patient age was 58.5 years (range, 28–74 years). Eighteen patients had PS 0, and the remaining 20 had PS 1. The primary tumor was located in the colon in 23 patients (61%) and in the rectum in 15 patients (39%). Thirty-three patients (87%) had well or moderately differentiated adenocarcinoma, whereas 5 (13%) had poorly differentiated adenocarcinoma. Of the 38 patients in the current study, 29 (76%) had undergone surgery before entry, 4 (11%) had received 5-FU-based adjuvant chemotherapy, and 2 had received pelvic radiotherapy.

The mean BSA in the current study population was 1.53 m^2 (range, 1.26–1.85 m^2). Daily S-1 doses according to BSA are shown in Table 2. The median S-1 dose was 60 mg administered twice daily. A combined total of 173 treatment courses were administered to the 38 patients enrolled in the study. The median number of courses per patient was 3.5 (range, 1–18), and the median cumulative S-1 dose per patient was 10,080 mg (range, 2660–44,660 mg).

Response

All 38 patients had measurable metastatic lesions. Although no patient experienced a CR, 15 patients had PRs (response rate, 39.5%; 95% confidence interval, 24.0–56.6%) (Table 3). Among these 15 patients, the median time required for a 50% reduction in tumor

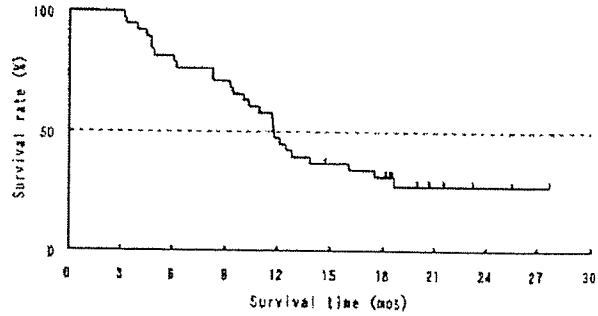

FIGURE 1. Overall survival of 38 patients treated with S-1 for previously untreated metastatic colorectal carcinoma. Median survival time, 358 days (95% confidence interval, 305–490 days).

size was 68 days (range, 29–130 days), and the median duration of response was 232 days (range, 96–679 days). Five patients had minor responses, and 14 had SD. The remaining four patients were found to have PD after two courses of treatment. Response rates according to metastatic site were as follows: liver, 38% (9 of 24 patients); lung, 27% (4 of 15 patients); and lymph nodes, 30% (3 of 10 patients). The response rate among patients with colon carcinoma was 44% (10 of 23 patients), and the response rate among patients with rectal carcinoma was 33% (5 of 15 patients). The response rate at the primary site as evaluated using the roentgenographic evaluation criteria proposed by the Japanese Society for Cancer of the Colon and Rectum was 43% (3 of 7 patients). One of the four patients who had a history of adjuvant chemotherapy achieved a PR.

At the close of the trial, the median time to evidence of disease progression was 162 days (range, 118–254 days). The median survival time from the beginning of treatment was 358 days (median follow-up, 666 days; 95% confidence interval, 305–490 days) for the overall study cohort, and the 1-year survival rate was 47.4% (Fig. 1).

Toxicity

For each toxicity, the patient distribution with respect to highest observed grade is summarized in Table 4. The most common adverse reactions included myelosuppression and gastrointestinal toxicity, although these events generally were mild, and no cumulative toxicity was noted. Neither Grade 4 toxicity nor treatment-related death was observed during the study. Toxicity incidence rates were as follows: anemia, 45% (17 of 38 patients); leukopenia, 45% (17 of 38 patients); neutropenia, 42% (16 of 38 patients); and thrombocytopenia, 13% (5 of 38 patients). Nonetheless, Grade ≥ 3 toxicities were noted in less than 8% of patients.

TABLE 4
Toxicity Data

Toxicity	Grade 1	2	3	4	Grade ≥3 (%)
Anemia	7	7	3	0	7.9
Leukopenia	7	10	0	0	0
Neutropenia	4	10	2	0	5.3
Thrombocytopenia	4	1	0	0	0
Diarrhea	5	8	1	0	2.6
Nausea/vomiting	8	7	0	0	0
Anorexia	15	4	0	0	0
Stomatitis	11	3	0	0	0
Hand-foot syndrome	2	0	0	0	0
Pigmentation	15	0	0	0	0
Malaise	17	2	0	0	0
Bilirubinemia	—[a]	14	3	0	7.9

[a] Grade 1 bilirubinemia is not defined in the toxicity criteria of the Japan Society for Cancer Therapy. (See: Japan Society for Cancer Therapy. Criteria for the evaluation of the clinical effects of solid cancer chemotherapy. *J Jpn Soc Cancer Ther.* 1993;28:101–130.[26])

The overall incidence rate for diarrhea was 37% (14 of 38 patients), with Grade 3 diarrhea noted in 3% of the study cohort (1 of 38 patients). The overall stomatitis incidence rate was 37% (14 of 38 patients); however, Grade ≥ 3 stomatitis was not observed. The incidence rate for hand-foot syndrome (palmar-plantar erythrodysesthesia) was 5% (2 of 38 patients); Grade 1 erythrodysesthesia was noted in both cases. Overall, abnormal bilirubin levels were noted in 45% of the study cohort (17 of 38 patients), with an incidence rate of 8% (3 of 38 patients) for Grade 3 bilirubin abnormalities. Nonetheless, no Grade ≥ 3 elevation of aspartate aminotransferase or alanine aminotransferase levels was observed in the current study.

Toxicity caused two patients to discontinue S-1 treatment. One of these two was hospitalized for abdominal pain (Grade 2), nausea with vomiting (Grade 2), and anorexia (Grade 2) during the third treatment course, and S-1 treatment subsequently was discontinued. The other patient withdrew from the study during the second treatment course due to diarrhea (Grade 3) and neutropenia (Grade 2). Discontinuation of treatment was not considered necessary for any of the other patients who experienced Grade 2 or Grade 3 toxicities; instead, these patients were able to continue receiving treatment after a brief interruption or after dose reduction. Thirty-five of 38 patients (92%) were treated as outpatients, a finding that indicates extremely good compliance. Of the 173 courses that were administered overall, 163 (94%) were administered at ≥ 75% of the protocol-defined dose.

DISCUSSION

The current study was conducted to evaluate the objective response rate and toxicity associated with an oral regimen of S-1 for patients with previously untreated metastatic colorectal carcinoma. We observed a response rate of 39.5%, which was equal to or greater than the corresponding response rates associated with 5-FU alone and with 5-FU + LV. In an earlier Phase II study of S-1, an overall response rate of 35% was reported for patients who had not previously received chemotherapy.[24] That earlier study and the current one were similar in terms of dosing and scheduling of S-1, eligibility criteria, and response criteria, and both studies also reported similar response rates and survival times; these similarities suggest that the activity of oral S-1 against metastatic colorectal carcinoma represents a reproducible finding.

In a previous Phase I study involving Japanese patients, S-1 was administered orally for 28 consecutive days.[22] The maximum allowable S-1 dose was 150 mg once daily or 75 mg twice daily, and myelosuppression (primarily leukopenia) was found to be the dose-limiting toxicity. This daily dose of 150 mg per day is equivalent to 100 mg/m^2 per day for the average Japanese patient, who has a BSA of 1.5 m^2. For the current study, we selected an S-1 dose of 80 mg/m^2 per day (40 mg/m^2 twice daily), which was slightly less than the maximum allowable dose identified by Phase I trials.[22] The most commonly observed adverse reactions in the current study were myelosuppression and gastrointestinal toxicity; these events generally were mild, with no Grade 4 toxicity noted. Although a small number of cases of Grade 4 myelosuppression have been reported in other Phase II studies in which a total daily dose of 80 mg/m^2 S-1 was used to treat malignant disease (gastric,[14,15] colorectal,[24] head and neck,[16] lung,[27] or breast[28]), the incidence and degree of toxicity observed in those studies did not differ substantially from what was documented in the current study.

The toxicity profile of 5-FU is schedule dependent. Myelosuppression is the primary toxic effect observed in patients receiving bolus 5-FU schedules, whereas hand-foot syndrome, stomatitis, neurotoxicity, and cardiotoxicity are associated with continuous infusion of 5-FU.[7] Hand-foot syndrome, in addition to being a typical side effect of prolonged 5-FU administration via CVI,[29] is commonly associated with the oral administration of other fluoropyrimidines, such as capecitabine.[10,11] The mechanism involved in the development of hand-foot syndrome has not been completely elucidated; however, some 5-FU catabolites are believed to be inducers of this condition.[30]

Thus, the low incidence of hand-foot syndrome associated with UFT use is consistent with the observation of low plasma levels of 5-FU catabolites in patients receiving UFT.[31] In the current trial, hand-foot syndrome was observed in only 5% of the study cohort (2 of 38 patients); furthermore, both of these cases involved reversible, Grade 1 hand-foot syndrome. In other trials, only mild S-1-induced hand-foot syndrome, which was not suggestive of dose-limiting toxicity, has been reported. These findings may reflect the inhibitory effect of CDHP on DPD.

The pharmacokinetic characteristics of prolonged S-1 administration were believed to be consistent with the use of CVI; however, the dose-limiting toxicity induced by S-1 was myelosuppression, which is associated with the bolus dose protocol. In a previous Phase I study, the maximum plasma 5-FU concentration was estimated to be approximately 230 ng/mL for Japanese patients who received S-1 at a dose of 75 mg per day.[22] This relatively high peak plasma 5-FU concentration may result in myelotoxicity, rather than gastrointestinal toxicity, in spite of the prolonged S-1 administration protocol. The low severity of gastrointestinal toxicity, even in the face of a relatively high peak plasma 5-FU concentration[22,23] and area under the plasma concentration–time curve, suggests the usefulness (previously noted in rats[19]) of potassium oxonate in humans. The toxicity observed in the current trial, in which S-1 was administered at a dose of 80 mg/m^2 per day (40 mg/m^2 twice daily), was mild and reversible, and yet the observed activity was remarkable, being equal to or greater than the activity of 5-FU alone.

Oral chemotherapy, for which only limited hospitalization is necessary, has major advantages over intravenously administered treatment in terms of pharmacoeconomic considerations and patient preference, as well as compliance.[12] In one study, it was reported that more than 90% of patients with advanced solid malignancies preferred oral agents over infusional agents when both types of treatment provided comparable efficacy.[32] Furthermore, a randomized crossover trial involving patients with advanced colorectal carcinoma found that oral UFT + LV compared favorably with intravenous 5-FU + LV in terms of toxicity and patient preference.[31]

In the current study, the S-1 regimen was administered successfully, with good treatment compliance, on an outpatient basis. Due to the absence of severe toxicity, especially with regard to symptoms such as nausea, vomiting, and diarrhea, almost all patients received ≥ 75% of the full protocol-defined S-1 dose; it is clear that good compliance increases the likelihood of favorable therapeutic responses. Thus, the findings of the current study indicate that S-1 is a promising agent that has the potential to become a valuable oral treatment option, along with capecitabine and UFT + LV, for patients with colorectal carcinoma. Clinical studies of S-1 in the treatment of metastatic colorectal and gastric malignancies[33,34] also suggest that S-1 possesses superior therapeutic activity compared with other regimens.

The combination of irinotecan or oxaliplatin with 5-FU + LV recently has been identified as a candidate regimen for the standard treatment of metastatic colorectal carcinoma. To determine which of these chemotherapeutic agents are most suitable for use in combination with S-1, clinical trials are essential. Three Phase I/II trials of S-1 with LV irinotecan or oxaliplatin for the treatment of metastatic colorectal carcinoma have been scheduled. In addition, a Phase III study of adjuvant chemotherapy (surgery alone vs. surgery followed by S-1) in the treatment of gastric tumors and a Phase III study comparing the use of S-1 alone with the use of S-1 + cisplatin in the treatment of metastatic gastric malignancies are ongoing. In another ongoing Phase III trial involving patients with gastric malignancies, the Japan Clinical Oncology Group is comparing 5-FU, which currently is the standard treatment agent, with single-agent S-1 and with cisplatin + irinotecan.

REFERENCES

1. Scheithauer W, Rosen H, Kornek GV, Sebesta C, Depisch D. Randomized comparison of combination chemotherapy plus supportive care with supportive care alone in patients with metastatic colorectal cancer. *BMJ.* 1993;306:752–755.
2. Douillard JY, Cunningham D, Roth AD, et al. Irinotecan combined with fluorouracil compared with fluorouracil alone as first-line treatment for metastatic colorectal cancer: a multicentre randomized trial. *Lancet.* 2000;355:1041–1047.
3. Douillard JY, Sobrero A, Carnaghi C, et al. Metastatic colorectal cancer: integrating irinotecan into combination and sequential chemotherapy. *Ann Oncol.* 2003;14 Suppl 2:II7–II12.
4. Saltz LB, Cox JV, Blanke C, et al. Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. *N Engl J Med.* 2000;343:905–914.
5. Sargent D, Niedzwiecki D, O'Connell MJ, Schilsky RL. Recommendation for caution with irinotecan, fluorouracil, and leucovorin for colorectal cancer. *N Engl J Med.* 2001;345: 144–145.
6. Goldberg RM, Morton RF, Sargent DJ, et al. N9741: oxaliplatin (oxal) or CPT-11 + 5-fluorouracil (5FU)/leucovorin (LV) or oxal + CPT-11 in advanced colorectal cancer (CRC). Initial toxicity and response data from a GI Intergroup study [abstract]. *Proc Am Soc Clin Oncol.* 2002;21:128a.
7. Malet-Martino M, Martino R. Clinical studies of three oral prodrugs of 5-fluorouracil (capecitabine, UFT, S-1): a review. *Oncologist.* 2002;7:288–323.