Volume 39

Diseases of the
COLON & RECTUM

Number 10

OCTOBER 1996

ORIGINAL CONTRIBUTIONS

# Tumor Angiogenesis in Primary and Metastatic Colorectal Cancers

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PURPOSE: Angiogenesis is needed to sustain growth of both primary and metastatic lesions; however, comparisons in microvessel density between a primary tumor and its metastases have not been widely performed. We studied microvessel density in primary colorectal cancers and their liver metastases. METHODS: Sections from 32 primary lesions and 53 hepatic metastases were immunostained with a monoclonal antibody for von Willebrand's factor, an endothelial cell marker. Blood vessels were quantified under ×100 magnification using both conventional light microscopy and computer-assisted image analysis. Primary and metastatic angiogenesis scores (AS), *i.e.*, vessel counts, were analyzed with respect to tumor size, hepatic multicentricity, synchronicity, resectability, and patient survival. Using computer-assisted calculations, the same analyses were performed using blood vessel to tumor surface area ratios, vessel wall thickness, and intensity of immunostaining. RESULTS: Angiogenesis scores were significantly lower in metastatic lesions compared with their primary tumors ($P < 0.0001$). Primary AS did not correlate with metastatic tumor size, resectability, multicentricity, or patient survival. Metastatic AS strongly predicted patient survival ($P < 0.0009$) but with a negative coefficient, *i.e.*, higher scores were associated with improved survival. Metastatic AS were higher in resectable than in nonresectable metastases and in solitary than in multiple metastases; however, these trends were not statistically significant. Metachronous liver lesions had significantly higher angiogenesis scores than synchronous metastases ($P < 0.04$). Similar trends were seen using computer-assisted image analysis. CONCLUSIONS: These results indicate that in presence of an established metastasis, there is a weak angiogenic relationship between a primary tumor and its metastasis. Heterogeneity in metastatic lesions cannot be explained solely by studying angiogenesis in primary tumors. Microvessel density in a primary tumor may not be useful as an independent prognostic indicator in late stages of disease. In such cases, assessment of microvessel density in a metastatic tumor whenever possible may be an indicator of prognosis. [Key words: Angiogenesis; Colorectal cancer; Liver metastasis]

Mooteri S, Rubin D, Leurgans S, Jakate S, Drab E, Saclarides T. Tumor angiogenesis in primary and metastatic colorectal cancers. Dis Colon Rectum 1996;39:1073-1080.

Growth of solid tumors is dependent on angiogenesis, and influx of new blood vessels may facilitate bleeding and dissemination to distant sites.[1-4] Studies correlating the quantitative assessment of angiogenesis, and risk of metastasis have been performed for breast, bladder, and nonsmall-cell lung cancers.[4-10] We found that there is significant association between vessel counts and both tumor depth of penetration and survival for patients with rectal cancers.[11] We also showed that a quantitative angiogenesis score is a very strong predictor of recurrence and survival following resection of node-negative colon cancers.[12]

Angiogenesis is a necessary prerequisite for growth of secondary tumors, many of which are initially inapparent clinically.[13, 14] Most often, these grow faster than their primary tumors, and this rapid growth may hasten patient demise and decrease survival times. A quantitative study comparing angiogenesis in primary

Funded by the Bowman Research Fund.
Read at the meeting of The American Society of Colon and Rectal Surgeons, Montreal, Quebec, Canada, May 7 to 12, 1995.
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lesions and their metastases may provide insight into tumor behavior and prognosis. The purpose of our study was to determine this relationship. We chose the liver because it is the most common site of metastases for colorectal cancer and is often resected for cure. Although it is known that resectable hepatic metastases fare better than those that are unresectable, we wanted to determine whether quantifying the degree of angiogenesis in the metastasis itself would be another prognostic indicator.

The current study, therefore, consisted of three separate but related questions. First, we wanted to determine if a quantitative assessment of angiogenesis in the primary tumor predicted patient survival (once metastases occurred) and whether its liver metastases would be solitary, metachronous, or resectable. Second, we sought to establish if angiogenesis scores within the metastasis itself reflected any of these growth patterns. Last, we wanted to determine if computer-assisted image analysis proved to be superior to conventional light microscopy as a means of studying angiogenesis. With commercially available computer software, not only can one count vessels but also one is able to obtain data, such as blood vessel/tumor surface area ratios and thickness of blood vessel walls, and calculate the optical density or intensity of staining by primary antibody.

## MATERIALS AND METHODS

Paraffin blocks of 53 colorectal hepatic metastases were retrieved. These were either resected for cure or biopsied, if unresectable, at Rush-Presbyterian-St. Luke's Medical Center, Chicago, Illinois, from 1984 to 1993. Of these, 32 patients had their primary tumors resected at Rush, and paraffin blocks from these tumors were also retrieved. Sections were taken only from nonnecrotic tumor-bearing areas, and one representative section per tumor was stained. After initial deparaffinization, each section was immunostained with a monoclonal antibody (Accurate Chemicals and Scientific Corporation, Westbury, NY, horseradish peroxidase rabbit antibody) to human von Willebrand factor-related antigen, a marker of endothelial cells. Standard streptavidin-labeled avidin-biotin technique (DAKO, Carpenteria, CA) was used as described previously.[15] Endogenous biotin activity of liver tissue was blocked by incubation with avidin-biotin blocking system (DAKO) which contained 1 percent avidin and 1 percent biotin, just before application of blocking antibody.[16] After staining, blood vessels appeared intensely red in color, which facilitated identification and quantification. Representative sections of primary colorectal and metastatic liver tumors stained for F-VIII are seen in Figure 1A and B.

Positive and negative controls were used to verify accurate staining methods. Positive control was performed on tonsillar tissue that is highly specific for primary antibody. This step ensured that the entire sequence of steps from fixation to staining was correctly performed. Negative control omits use of primary antibody, substituting instead rabbit serum, and is used to evaluate for the presence of nonspecific staining. Positive and negative controls were run for every ten tumors sectioned and stained. Several normal sections obtained at autopsy (patients died of causes other than colorectal cancer) were also stained and used as a positive control.

Microvessel counts were determined without knowledge of the pathology report or patient outcome. The angiogenesis score of each slide was determined by using a phase contrast microscope at ×100 magnification. Initially, a subjective angiogenesis grade was determined by scanning the entire slide

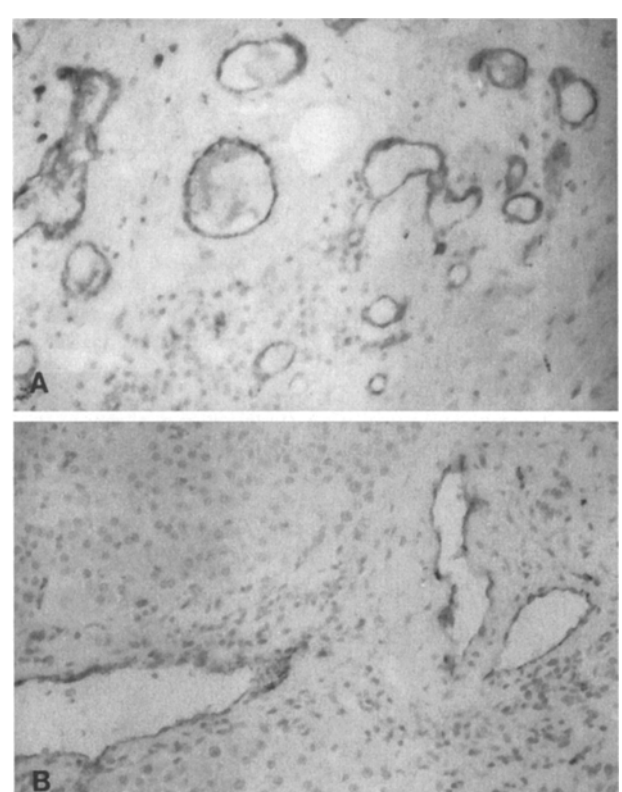


**Figure 1.** A. von Willebrand factor-related antigen staining of primary colorectal cancer. B. von Willebrand factor-related antigen staining of liver metastasis from colorectal cancer.

and assigning a grade from 1 to 4, depending on vascularity. Then three fields with areas of intense vascularization at the periphery of the tumor were considered for blood vessel quantification. A 1 × 1 cm, microscopic grid was placed within the ocular eyepiece of the microscope, and total number of blood vessels within the unit area of the grid was counted. Any blood vessel projecting more than one-half into the grid area was counted. Doubtful areas were not counted; any vessel seen on cross-section or any tangential section that was isolated from other vessels was counted as a single vessel. Clusters of tortuous blood vessels in which one lumen could not be distinguished from another were counted as a single vessel. Any red-staining endothelial cell or endothelial cluster, clearly separate from adjacent microvessels, tumor cells, and stromal elements was also counted as a single vessel. The number of definable blood vessels in each field was tabulated separately, and the mean was calculated. In liver metastases, sinusoids did not stain for von Willebrand factor-related antigen and, hence, were not counted.

Pathology reports and records of our tumor registry were then reviewed in a retrospective fashion. Tumor size was determined by original pathologic material. Liver angiogenesis scores (LAS) were calculated (n = 53) and assessed with respect to liver tumor size, its resectability, multicentricity, synchronicity, carcinoembryonic antigen levels, presence of extrahepatic sites, and patient survival. Tumor size was considered only in resected liver tumors (n = 30) because nonresected lesions were only biopsied; and those instances in which multiple nodules were resected, mean tumor size was used for calculations. In 32 patients, angiogenesis in the colon tumor (CAS) was compared with its corresponding LAS, and we sought to determine whether CAS predicted secondary tumor size, its resectability, multicentricity, synchronicity, or patient survival.

Computer-assisted analyses used a system with a video camera to capture the image, a capture board that digitally converts the analog video signal and directs the digital data to a computer that performs a variety of software-driven operations, a video monitor, and storage media. This type of quantitative imaging makes use of gray scale brightness (luminescence), providing 256 levels of gray. The system is interactive, requiring operator input to include or exclude objects for analysis or cut between adjoining objects while other functions are automated.

Total blood vessel to tumor surface area ratio and vessel wall thickness were measured. The area of the monitor screen was considered as a constant tumor surface area. Individual vessel surface area was determined by outlining each vessel with the mouse, the total vessel surface area summated, and the ratio automatically calculated.

Intensity of von Willebrand factor-related antigen staining was determined by measuring optical density. Strength of the video signal at any particular location is linearly proportional to the light transmitted at that point. Optical density (OD) is defined as the negative logarithm of the ratio of the transmitted light to the incident light and is measured from an adjacent clear area of the slide; its value is established and standardized to zero before measurements are taken. According to the Boer-Lambert law, mass of the absorbing substance at any point is linearly proportional to the OD. Therefore, total OD of all pixels can be used to measure the quantity of cellular components.

## Statistical Methods

Components of variance were used to assess for any differences within a tumor or between tumors using random effect models. Standard descriptive statistics were obtained for all variables. Two sample *t*-tests and one-way analysis of variance were used to compare mean angiogenesis scores (AS) of groups of patients. Linear regression was used to determine whether tumor sizes predicted angiogenesis scores. Logistic regressions of clinical outcomes on angiogenesis scores and on tumor sizes were obtained. Spearman's rank-correlation coefficients were obtained to measure association of angiogenesis score and angiogenesis grade. Survival curves and disease-free interval were estimated using Kaplan-Meier product limit estimators. Log-rank tests were used to compare distribution of times of groups of patients. Univariate Cox proportional hazards regression was used to examine the relationship of continuous variables to event times. These analyses were supplemented by examination of three-year survival and one-year disease-free interval *via* chi-squared tests, Fisher's exact tests when appropriate, and logistic regression (likelihood ratio tests). Computations were performed using SAS® 6.07 (SAS Institute Inc., Cary, NC) and S-Plus™ (Statistical Sciences, Inc., Seattle, WA) on a Sun™ SPARKstation 10™ (Sun Microsystems, Inc., Mountain View, CA). All significance levels are for two-tailed tests; statistical significance was indicated by $P < 0.05$.

## RESULTS

Analysis of variance of angiogenesis scores among three different fields within a tumor showed excellent reproducibility and little variance both in primary and secondary tumors. Subjective assessment of angiogenesis grade correlated well with quantitative scores ($P < 0.0001$).

### Hepatic Metastasis (n = 53)

The relationship between LAS and the different variables are shown in Table 1. Normal liver tissue had a mean LAS of 3.83 ± 924. For the entire group of metastases, mean LAS was 9.46 ± 6.54. LAS did not differ significantly by sex and did not correlate with tumor size. Also, tumor sizes either alone or by classes did not vary significantly by vessel number. Higher LAS were found in resectable than in nonresectable lesions and in solitary than in multiple lesions, but neither difference reached statistical significance. Significantly higher angiogenesis scores were seen in metachronous tumors compared with synchronous tumors ($P = 0.04$). No significant correlation was found between LAS and carcinoembryonic antigen levels or presence of extrahepatic disease.

### Primary Tumor *vs.* Metastatic Tumor (n = 32)

Table 2 summarizes comparison between primary and metastatic angiogenesis scores. Primary AS had a positive correlation with metastatic AS ($r = 0.25$). LAS were significantly lower than their primary counterparts ($P < 0.0001$). Neither size (primary or secondary) was associated with a significantly different mean score for either site. Primary tumor size and CAS did not predict liver tumor size, number of nodules, resectability, or synchronicity. Although CAS was higher with nonresectable than resectable liver metastases, in multiple than in solitary metastases, and in synchronous than in metachronous liver lesions, none of the trends reached statistical significance. Similarly, there were no significant differences in corresponding LAS. Overall, CAS did not correlate with any specific metastatic growth pattern.

### Survival Analysis

Survival curve from time of diagnosis of metastatic disease to death was obtained by using Kaplan-Meier estimates. Median survival after diagnosis of hepatic metastasis was 22 months (Fig. 2).

**Table 1.**
Conventional Light Microscopy

| Characteristics | n | Mean ± SD | P |
|---|---|---|---|
| Sex | | | |
| Female | 22 | 10.93 ± 8.12 | |
| Male | 31 | 8.47 ± 5.03 | 0.18 |
| Size of liver tumor (cm)* | | | |
| <4 | 17 | 9.65 ± 8.35 | |
| 4–6 | 7 | 12.31 ± 6.81 | |
| >6 | 6 | 11.83 ± 5.10 | 0.679 |
| Resectability | | | |
| Nonresectable | 23 | 7.90 ± 4.84 | |
| Resectable | 30 | 10.71 ± 7.36 | 0.12 |
| Multicentricity | | | |
| Multicentric | 36 | 8.88 ± 6.71 | |
| Solitary | 17 | 10.78 ± 5.95 | 0.32 |
| Synchronicity | | | |
| Metachronous | 22 | 11.92 ± 8.06 | |
| Synchronous | 31 | 7.77 ± 4.48 | 0.02 |
| Extrahepatic Sites | | | |
| Absent | 39 | 9.74 ± 7.19 | |
| Present | 14 | 8.79 ± 4.04 | 0.64 |
| Multicentricity by resectability† | | | |
| Solitary Resectable | 17 | 10.78 ± 5.95 | |
| Multicentric, Resectable | 13 | 10.62 ± 9.14 | |
| Multicentric, Nonresectable | 23 | 7.90 ± 4.84 | 0.30 |
| Synchronicity by resectability† | | | |
| Metachronous, Nonresectable | 3 | 3.89 ± 1.17 | |
| Metachronous, Resectable | 19 | 13.18 ± 7.94 | |
| Synchronous, Nonresectable | 20 | 8.50 ± 4.90 | |
| Synchronous, Resectable | 11 | 6.44 ± 3.41 | .007 |

SD = standard deviation.

Angiogenesis score in hepatic metastases = 53; normal liver angiogenesis score = 3.83 ± 0.24; mean angiogenesis score in hepatic metastases = 9.46 ± 6.54.

* Angiogenesis scores shown are for resectable lesions. If more than one lesion was resected, the mean tumor size was used for the calculations.

† Analysis of variance.

Three-year survival was obtained by cross-sectional analysis. Three-year survival was studied because only one person changed status between years 3 and 4, whereas others were not at risk for the entire five years. Patients who died in the postoperative period and those who were alive with less than three years follow-up were excluded from the analysis, leaving 38 patients. In general, patients with unresectable tumors fared significantly worse ($P = 0.03$); no one was alive

**Table 2.**
Conventional Light Microscopy

| | n | Colon | | Liver | |
|---|---|---|---|---|---|
| | | Mean ± SD | *P* | Mean ± SD | *P* |
| AS | 32 | 16.69 ± 9.01 | | 9.46 ± 6.54 | <0.0001 |
| Tumor size (colon) (cm) | | | | | |
| <4 | 10 | 16.96 ± 11.38 | 0.56 | | |
| 4–6 | 15 | 19.57 ± 7.31 | | | |
| >6 | 7 | 14.70 ± 5.6 | | | |
| Liver resectability | | | | | |
| Nonresectable | 20 | 18.37 ± 4.84 | 0.22 | | |
| Resectable | 12 | 14.35 ± 5.95 | | | |
| Liver multicentricity | | | | | |
| Multicentric | 24 | 17.38 ± 9.84 | 0.57 | | |
| Solitary | 8 | 15.17 ± 6.09 | | | |
| Liver synchronicity | | | | | |
| Metachronous | 4 | 14.74 ± 6.00 | 0.63 | | |
| Synchronous | 28 | 17.12 ± 9.42 | | | |

AS = Angiogenesis Score; SD = standard deviation.
P = 0.04 by unequal variance *t*-test.
* Primary *vs.* metastatic AS n = 32. ANOVA (analysis of variance).

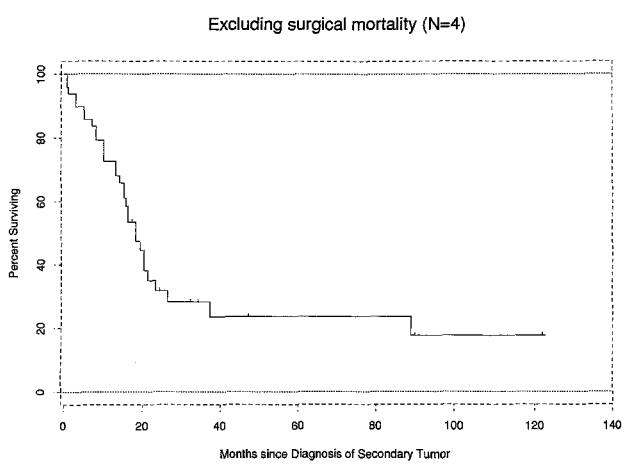


**Figure 2.** Survival from diagnosis of liver metastases, all patients (n = 53).

**Table 3.**
Cross-Sectional Analysis of Three-Year Survival

| Variable | Survival (%) | *P* Value* |
|---|---|---|
| Synchronicity | | |
| Metachronous (16) | 31 | 0.06 |
| Synchronous (22) | 5 | |
| Resectability | | |
| Nonresectable (16) | 0 | 0.03 |
| Resectable (22) | 27 | |
| Multicentricity | | |
| Multiple (26) | 4 | 0.008 |
| Solitary (12) | 71 | |

* Fisher's exact test.

more than three years after diagnosis, whereas 27 percent of patients with resectable lesions were alive at three years. A significantly lower survival rate was seen in patients with multicentric liver tumors ($P$ = 0.008). Patients with synchronous disease did worse, but the comparison was of borderline significance ($P$ = 0.0657; Table 3).

## Angiogenesis Scores and Survival

Univariate Cox proportional hazards regression showed significance only for LAS ($P$ = 0.05) but with a negative coefficient (−0.0573). By logistic regression analysis of survivors, LAS strongly predicted the probability of being alive ($P$ < 0.0009) at three years, but again with a negative coefficient (−0.27), *i.e.*, patients with higher angiogenesis scores survived longer. Colon angiogenesis scores did not predict patient survival either by Cox proportional hazards regression or logistic regression.

## Computer-Assisted Analysis

Corresponding $P$ values using data generated by computer-assisted analysis are shown in Table 4, and results obtained by conventional light microscopy were verified. In addition, calculations of the blood vessel/tumor surface area ratio and measurements of blood vessel wall thickness among the variables tested (multicentricity, synchronicity, etc.) followed similar trends as the AS. Assessment of staining inten-

**Table 4.**
Computer-Assisted Analysis: Hepatic Metastasis (analysis of variance)

| Variable | n | AS | | Log Area Ratio | | Log Vessel Thickness | | Log Optical Density | |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean ± SD | *P* | Mean ± SD | *P* | Mean ± SD | *P* | Mean ± SD | *P* |
| Resectability | | | | | | | | | |
| Nonresectable | 21 | 7.33 ± 4.39 | 0.035* | 1.11 ± 1.32 | 0.017* | 1.56 ± 0.41 | 0.14 | −1.68 ± 0.51 | 0.9 |
| Resectable | 29 | 12.06 ± 9.21 | | 1.86 ± 0.8 | | 1.75 ± 0.36 | | −1.69 ± 0.48 | |
| Multicentricity | | | | | | | | | |
| Multicentric | 34 | 9.58 ± 7.92 | 0.5 | 1.49 ± 1.21 | 0.5 | 1.61 ± 0.39 | 0.054 | −1.74 ± 0.54 | 0.3 |
| Solitary | 16 | 11.1 ± 7.9 | | 1.70 ± 0.82 | | 1.84 ± 0.34 | | −1.58 ± 0.35 | |
| Synchronicity | | | | | | | | | |
| Metachronous | 22 | 13.0 ± 10.0 | 0.016* | 1.92 ± 0.84 | 0.045* | 1.85 ± 0.37 | 0.006* | −1.62 ± 0.43 | 0.4 |
| Synchronous | 28 | 7.74 ± 4.51 | | 1.29 ± 1.19 | | 1.55 ± 0.35 | | −1.74 ± 0.53 | |

SD = standard deviation; AS = angiogenesis score; Area Ratio = total blood vessel to tumor surface area ratio; Vessel Thickness = vessel wall thickness; Optical Density = intensity of staining.
* Significant *P* values, $P < 0.05$.

**Table 5.**
Computer-Assisted Analysis of Hepatic Blood Vessels

| Variable | Vessel Type | n | Liver | |
|---|---|---|---|---|
| | | | Mean ± SD | *P* value |
| Area ratio | Large | 26 | 2.03 ± .84 | 0.0007 |
| | Small | 24 | 1.02 ± 1.11 | |
| Angiogenesis score | Large | 26 | 6.44 ± 5.15 | 0.0003 |
| | Small | 24 | 14.01 ± 8.5 | |
| Vessel wall thickness | Large | 26 | 4.67 ± 1.37 | 0.0002 |
| | Small | 24 | 6.84 ± 2.33 | |

sity uniformly failed to vary among the various factors.

In addition to the above features, we also noted two distinct morphologic varieties of blood vessel patterns in liver metastases only. We called these predominantly small vessel or large vessel type depending on size. Area ratios and vessel counts of these vessel types were determined separately (Table 5). Multicentric lesions had a significantly higher number of large vessels (80 percent; $P < 0.04$); however, there was no significant difference between the two vessel types with regard to resectability or synchronicity.

## DISCUSSION

Cancer dissemination is a complex multistep process involving many host-tumor interactions. Presence of tumor cells in the circulation coincides with onset of neovascularization.[13] As with the primary tumor, angiogenesis is necessary for growth and establishment of a metastatic deposit, which may then give rise to additional metastases. Thus, angiogenesis is necessary for both the beginning and end of the metastatic cascade.[9, 17, 18]

There are numerous studies that have correlated high microvessel counts with the invasive nature of a malignant tumor and its risks of metastasis and recurrence. In breast cancer, areas with the highest microvessel counts appear to contain the highest percentage of angiogenic cells.[10] However, it is not known whether the angiogenic potential of a primary tumor is maintained in its metastasis, because it has to overcome many other obstacles to thrive. In fact, to what extent the behavior and angiogenesis of a secondary tumor is determined by degree of angiogenesis in the primary tumor has not been widely studied.

This study focuses on two aspects of a metastatic lesion, the degree to which secondary tumor AS predicts behavior and survival and the extent of its association with the microvessel density in its primary tumor. We chose to study liver metastases because they occur commonly in the natural history of colorectal cancer, and frequently there is the opportunity to obtain tissue either in attempting curative resec-

tions or simply in biopsying in the face of unresectable disease.

Often a metastatic tumor grows faster than the primary lesion. Occasionally an occult primary can have a very large metastatic tumor; in these instances, one might expect more angiogenesis in the secondary than in the primary tumors. Surprisingly, our results indicated the opposite. This observation may be attributable to the different cellular environment that the liver provides; namely, its increased vascularity and rich energy stores. As such, ingrowth of new blood vessels may not be needed to the same degree as in the primary site to sustain increased tumor growth. Furthermore, organ-derived growth factors may regulate tumor growth and angiogenesis in ways unique to that particular organ.[19] It has also been suggested that increased vascularity of the liver combined with its relatively lower amount of connective tissue effectively disperse angiogenic stimuli away from endothelial cells.[20] It is reasonable to assume that low relative hepatic microvessel density observed in the present study may be multifactorial. Further studies are needed to confirm that the degree of angiogenesis is organ-specific.

We did not find significant differences in liver angiogenesis scores between different tumor sizes, resectable or nonresectable and solitary or multiple lesions. Significantly higher scores were found in metachronous lesions than in synchronous lesions. Although we do not have a satisfactory explanation, this may be related to lead-time bias and time of diagnosis of the secondary tumor during its growth period. Metachronous lesions may not be found until they reach a size large enough for detection or produce distressing symptoms. Alternatively, synchronous lesions are usually found at laparotomy for the primary tumor, and the degree to which they impact on clinical presentation or symptomatology and potentially on neovascularity may be less significant than in the case of metachronous lesions. Also, synchronous lesions may grow faster and produce necrosis that may account for their low microvascular density.

We compared the angiogenesis score and size of primary tumor with its corresponding hepatic metastasis. Neither the angiogenesis score nor the size of the primary tumor predicted the behavior of the secondary lesion in terms of its resectability, multicentricity, or synchronicity. However, our results did indicate a weak positive correlation between angiogenesis scores of the primary and its secondary. A similar but significant positive correlation between blood capillary density ratio of a primary prostatic cancer and its bone marrow metastasis was reported earlier.[21] Only a detailed study of cell biology and kinetics of both the primary and its secondary tumor would confirm whether this is an indication of inheritance of phenotypic expression of an angiogenic potentiality from the primary tumor.

Prognostic significance of angiogenesis in a metastatic lesion was not studied before our work, and it is not known whether antiangiogenic therapy is useful even in early metastasis. In the present study, microvessel density in the liver tumor strongly predicted three-year survival, although inversely; this may be related to biology of the metastasis itself. At the same time, in patients with an established metastasis, microvessel density in the primary tumor failed to predict prognosis.

Computer-assisted analysis may provide a uniform means of studying angiogenesis between examiners and institutions, but little additional information is gained beyond counting with a conventional light microscope. Intensity of immunostaining did not vary enough to provide meaningful insight into tumor biology. Similarly, area ratios and blood vessel thickness followed the same trends as simple vessel counts.

We observed two vessel types within metastatic lesions. Lesions with large vessels had lower angiogenesis scores and thinner walls. Two morphologic types of angiogenesis have been reported in experimental liver metastases,[20] a dominant sinusoidal type with large convoluted blood vessels lacking a basement membrane, and a portal type with numerous small vessels staining positive for basement membrane. Although we noted similar findings, the exact significance still needs to be determined, and it may become useful for targeting antiangiogenic therapy.

## CONCLUSION

This study demonstrates a weak association between a primary tumor and its metastasis with regard to degree of angiogenesis; furthermore, angiogenesis in the primary tumor does not predict behavior or growth patterns of the secondary. This study also confirms the fact that microvessel density in the primary tumor is useful in predicting prognosis only in the early stages before onset of disseminated disease. Quantitative assessment of angiogenesis in metastatic

lesions significantly correlated with survival; however, vessel counts were significantly different only in metachronous lesions. This observation may be influenced by lead-time bias. Vessel counts did not significantly vary by multicentricity or resectability of liver metastases.

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