Association of Angiogenesis Markers With Lymph Node Metastasis in Early Colorectal Cancer

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Hypothesis: We hypothesized that p53 mutations (mp53) are associated with decreased expression of thrombospondin 1 (TSP-1) and that decreased TSP-1 expression is associated with lymph node metastases.

Design: A retrospective study of lymphatic mapping and pathologic determination of angiogenesis markers in primary colorectal cancer.

Setting: Tertiary care cancer institute.

Patients: Sixty-one patients with colorectal cancer underwent lymphatic mapping. Lymph nodes that stained negative by hematoxylin-eosin were examined with immunohistochemistry for micrometastases. Primary tumors were analyzed by immunohistochemistry for mp53 and TSP-1 expression. The t test and the Mann-Whitney U test were used to examine the mean difference in TSP-1 expression between tumors.

Main Outcome Measures: Mutant p53 expression, TSP-1 expression, and metastatic progression.

Results: Thirty-six of the 61 patients (59%) had nodal metastases shown by hematoxylin-eosin or immunohistochemistry in the sentinel node (N2, N1, N1mi, or N0[i+]). Patients with a truly negative sentinel node (pN0[i−][sn]) had significantly higher TSP-1 expression compared with those with some degree of nodal metastases (57.7 vs 30.1; P < .001). Acquisition of mp53 was associated with a decreased mean TSP-1 expression. Tumors without mp53 expression had a mean TSP-1 optical density value of 51.3 while tumors with elevated mp53 had a mean TSP-1 optical density value of 31.8 (P < .03).

Conclusions: Patients with primary colorectal cancer with low TSP-1 expression, with or without detection of mp53 gene product, are more likely to harbor lymph node metastasis than patients with higher expression. Patients with a truly negative sentinel node (pN0[i−][sn]) frequently have higher expression of TSP-1 that may have inhibited metastatic progression. Further studies will investigate the relationship between mp53, TSP expression, and disease progression.

Arch Surg. 2007;142(8):738-745

C OLORECTAL CANCER (CRC) is the second leading cause of cancer-related death in the United States, with an estimated 148,610 new cases and 55,170 related deaths in 2006.1 Currently, lymph node metastasis and depth of the primary tumor are the most important prognostic factors predicting recurrence and disease-specific survival. Approximately 39% of patients will present with node-negative localized CRC. Traditionally, the majority of the node-negative individuals have been treated by observation alone, despite the fact that up to 30% eventually die of metastatic disease.2 Identifying high-risk patients with stage II colon cancer who may benefit from 5-fluorouracil–based chemotherapy has been difficult, and once a node-negative patient develops recurrence after observation alone, the opportunity for curative treatment may be lost. Such node-negative patients who truly benefit from chemotherapy still need to be identified.3

As more recent chemotherapeutic agents become more effective in treating stage III CRC (eg, oxaliplatin), the use of such treatment may increase in node-negative patients. If these subsets of patients who will benefit from chemotherapy remain elusive, many patients will be overtreated. Low-risk candidates who may avoid adjuvant chemotherapy might be identified by more accurate nodal assessment in combination with prognostically meaningful functional characteristics of the primary tumor.

One such group of functional markers are the angiogenesis markers. These markers, including p53 and the thrombospondins, are expressed by the primary tumor and are of particular interest because they have tumor-suppressive effects that have
been used to predict tumor biology in melanoma, breast, and prostate cancer. The p53 protein, in its nonmutated state, responds to cellular oncogenic stress in a protective way. After pathway activation, the p53-mediated response can result in cell-cycle arrest and apoptosis. The p53 protein can lose its function and subsequent downstream effects after the tumor acquires a p53 mutation (mp53) or when proteins that facilitate its stabilization are absent (eg, ARF protein).

Although the exact mechanisms of p53 are not clear, there are several complex pathways that regulate its activation, and once the protein becomes mutated, there are multiple changes that can occur in the tumor microenvironment. One of the changes associated with mutations in oncogenes and tumor suppressor genes is the decreased expression of another protective protein, thrombospondin 1 (TSP-1). Thrombospondin 1 is a member of a family of thrombospondins, glycoproteins that regulate tissue growth and remodeling. The effects of these proteins have been investigated in inflammation, wound healing, and neoplasia. In the tumor microenvironment, the interaction of TSP-1 with cell-surface receptors can lead to suppression of tumor growth through a wide range of effects including the modulation of cytoskeletal organization, cellular adhesion, migration, and apoptosis. Although a causal effect has not been definitively determined, in many tumors the decreased expression of thrombospondins is commonly seen in association with or after activation of oncogenes or inactivation of tumor suppressor genes (eg, mp53). Once the TSP-1 expression is decreased, studies suggest there is a disruption in the balance of angiostatic and angiogenic factors leading to more rapid tumor progression and the overall shift toward a proangiogenic environment.

Many angiogenesis markers have been studied in CRC but have not been used in conjunction with sentinel node status. Intraoperative lymphatic mapping identifies the sentinel lymph node(s) in the regional lymphatics (eg, mesentry) and has been associated with high overall nodal yields in CRC. This technique improves the accuracy of nodal assessment by allowing the pathologist to focus extra effort on the 1 to 4 nodes with the highest potential to harbor metastasis. Our group introduced this technique initially in melanoma and breast cancer to avoid completion lymphadenectomy and to improve staging. In CRC, we have begun using sentinel node mapping in addition to complete lymphadenectomy to detect micrometastatic nodal metastases (tumor cell clusters measuring ≤2 mm) missed by conventional methods alone; the extent of the operation is never limited. We have previously demonstrated that focused pathologic examination of the sentinel node(s) improves staging of CRC. Although the biologic significance of micrometastatic disease is unclear, recent meta-analysis assessing the clinical relevance of lymphatic micrometastasis suggests this is a biologically significant event that can affect survival. This technique also affords a unique opportunity to study the biology and characteristics of primary tumors associated with truly node-negative disease (ie, without evidence of lymphatic micrometastasis).

Based on prior studies, we suspected the primary tumor expression of mp53 would correspond to lower TSP-1 expression because mp53 may play a central role in tumor progression by regulation of TSP-1 at the transcriptional level. We hypothesized that mp53 are associated with decreased expression of TSP-1 in CRC, and once the TSP-1 expression is determined to be low, the frequency of lymph node metastasis increases. To investigate this, we analyzed primary tumor expression of mp53 and TSP-1 and the nodal status of patients who underwent intraoperative lymphatic mapping in conjunction with surgical resection of primary CRC. In the future, these angiogenesis markers (mp53 and TSP-1) in conjunction with more accurate nodal staging (sentinel node multilevel sectioning with cytokeratin immunohistochemical staining or molecular testing) could possibly be used to select high-risk patients with stage II colon cancer who may benefit from chemotherapy or perhaps be used to identify a low-risk group who could safely avoid chemotherapy, thus avoiding the cost and toxic effects of overtreatment.

**METHODS**

The study samples were obtained from patients who underwent lymphatic mapping of primary CRC at the John Wayne Cancer Institute or at McLaren Regional Medical Center, an affiliate of Michigan State University. The availability of paraffin-embedded sentinel lymph node and primary tumor specimens from each patient was required for entry into this study. One hundred ninety-five specimens were analyzed in addition to standard pathologic processing; samples included 61 evaluable primary tumors (p53 and TSP-1) and 134 sentinel lymph nodes (step sectioning and staining) from 61 patients. Samples included specimens obtained from 1996 through 2005. We excluded patients for whom an adequate amount of paraffin-embedded specimens were not available and/or patients whose records were incomplete.

**INTRAOPERATIVE LYMPHATIC MAPPING**

The intent of CRC lymphatic mapping is to assist the pathologist in identifying the most likely lymph node(s) to harbor metastasis if present; the resected surgical specimen is never limited or compromised. The technical details of colorectal lymphatic mapping have been described in detail previously. In brief, at laparotomy or laparoscopy, the extent of the primary tumor was evaluated and the colon was gently mobilized without extensive dissection of lymphatic channels or blood vessels. Rectal carcinomas were mapped without violating the oncologic principles during the total mesorectal excision. After isolation of the tumor, 0.5 to 3 mL of 1% isosulfan blue dye (Lymphazurin; US Surgical Corp, Norwalk, Connecticut) were injected circumferentially around the tumor in the subserosal layer using a tuberculin syringe. An afferent lymphatic channel was visualized leading to 1 to 4 sentinel lymph nodes within the mesentery. The sentinel lymph nodes were then marked with a suture. A standard oncologic resection of the primary tumor and all draining regional lymph nodes was completed. The specimen was sent en bloc to the pathology department.

**PATHOLOGIC EXAMINATION**

All paraffin-embedded sentinel lymph node specimens were examined at the John Wayne Cancer Institute or at McLaren Regional Medical Center. Sentinel lymph nodes were serially sec-
tioned at 20- to 40-µm intervals and examined by hematoxylin-

eosin (H&E) and cytokeratin immunohistochemistry (IHC) to
detect micrometastatic disease, according to the technique de-
dscribed by Wiese et al. 24 Immunohistochemistry was only per-
formed on nodes that were H&E negative. The pathologist de-
determined TNM staging according to American Joint Committee
on Cancer 6th edition staging guidelines. 25 Micrometastasis
greater than 0.2 mm, with no focus greater than 2.0 mm, was
classified as pN1mi. When no metastatic cluster measured greater
than 0.2 mm, the micrometastases were classified as isolated
tumor cells (pNO[i−]).

ANGIOGENESIS MARKERS
OF THE PRIMARY TUMOR: p53 AND TSP-1

Tissue blocks of primary tumors were coded and sent to On-
cotech Inc (Irvine, California) for blinded (without clinical data)
IHC and digital image analysis of TSP-1 expression and mp53
protein. There are a variety of limitations in IHC detection of
mp53. Although the technique is simple, some mutations may
not be detected and cofactors required for p53 function are not
accessed. Immunohistochemical analyses of mp53 and TSP-1
were performed on the primary tumor specimen according to
previously published methods. 4,6 In brief, 5-µm tissue sec-
tions of paraffin-embedded tumor specimens were cut and
mounted on poly-l-lysine slides (Superfrost Plus; VWR Inter-
national, West Chester, Pennsylvania). The tissues were depa-
raffinized in HistoClear (National Diagnostics, Atlanta, Geor-
gia) for 15 minutes and then rehydrated by sequential washing
in progressively increasing aqueous ethanol solutions. For analy-
sis of mp53, antigen retrieval was achieved by boiling in ci-
trate buffer for 15 minutes. Thrombospondin 1 detection did
not require antigen retrieval. Endogenous peroxidase activity
was inhibited by 10-minute incubation in 3% hydrogen per-
oxide (Peroxide Block; BioGenex, San Ramon, California). Slides
were then washed in phosphate-buffered saline. Non specific
protein binding was abolished by treating the slides with nor-
mal goat serum (Protein Block; BioGenex) for 10 minutes, fol-
dowed by application of the primary antibody. Paraffin-
embedded MCF-40F cells were used as positive controls for
mp53 and TSP-1 staining. Control cell line expression was con-
firmed by Western blot analysis. 8 Each specimen had an ac-
companying negative control using nonimmune IgG. In our
study, mp53 expression in formalin-fixed, paraffin-embedded
tissue sections was detected using 1-hour incubation with the
DO-1 monoclonal antibody (Santa Cruz Biotechnology, Santa
Cruz, California; final concentration, 0.5 µg/mL), recognizing
positive immunostaining as mp53 with increased protein half-
life. Tissue sections were pretreated with CCS-1 (Cell Condi-
tioning Solution 1; Ventana Medical Systems, Tucson, Ariz-
ona), followed by routine immunostaining on a Ventana
BenchMark instrument using the diaminobenzidien/
horseradish peroxidase kit (Ventana Medical Systems) for sec-
ondary detection.

Detection of TSP-1 required incubation with 2 µg/mL of
mouse monoclonal anti-TSP-1 antibody clone P12 (Immu-
no-tech, Inc, Westbrook, Maine) for 30 minutes. Slides under-
going evaluation for TSP-1 expression by image analysis were coun-
terstained with methyl green to avoid spectral overlap with the
IHC stain. A certified pathologist determined the degree of stain-
ing on all IHC slides.

LIGHT MICROSCOPY

p53 Mutations are the most frequent genetic changes in hu-
man cancer and are associated with an overexpression of the
mp53 protein with prolonged half-life. Tissue sections with tu-
mor cells staining positive for mp53 were graded for intensity
according to the following scale: negative when less than 5%
of tumor cells displayed stain; 1+ for mild intensity; 2+ for
moderate intensity; 3+ when intensity was equal to positive
controls; and 4+ when staining was greater than positive
controls. The formula

\[
\text{Percentage of Cells Staining Positive} \times (\text{Staining Intensity} + 1)
\]

was used to calculate histoscores for p53 expression. Because
any overexpression of p53 suggests mutation, a histoscore greater
than 0 was considered positive for mp53 expression. 26

DIGITAL IMAGE ANALYSIS

Tumor expression of TSP-1 was measured by optical density
(OD) of stained tumor sections using the CAS 2000 image analy-
ysis work station (Becton Dickinson, San Jose, California), as
described previously. 4 A 2-component image channel net-
work was used to examine TSP-1 IHC staining: one channel
specifically enhanced the image of tissue that had been coun-
terstained with methyl green (to avoid spectral overlap with
the immunostaining) and the other channel was used to
calculate the density of the brown stain per tissue area to iden-
tify the portion of cells stained with antibodies to TSP-1. Units
of staining for image analysis were reported as the measure of
the OD of stained tumor sections under magnifications ×400.
For each specimen, a minimum of 10 fields were measured.

STATISTICAL ANALYSIS

Thrombospondin 1 values were considered as continuous vari-
ables. The expression of mp53 (histoscore) was considered as
dichotomous variable. The t test was used to assess the mean
difference in TSP-1 OD values between tumors. Additionally,
\( \chi^2 \) analysis was used to determine the difference in TSP-1 OD
values among tumors with and without nodal metastases. Since
the exact normal TSP-1 OD level for CRC has not been defini-
tively established, we used the TSP-1 OD level of 30 or less
reported by Mehta and colleagues 4 to denote decreased TSP-1
expression. P values < .05 were considered statistically signif-

RESULTS

PATIENT POPULATION

The mean age of the 61 patients was 70.5 years. There were
30 men and 31 women. Primary tumors were distributed in the
ascending colon (n = 35), the descending colon (n = 24), and rectum
(n = 2). Twenty-five patients had no evidence of lymph node
metastasis after standard H&E histologic evaluation of all nodes
and after IHC staining of the sentinel lymph nodes through mul-
tiple sections (pNO[i−][sn]), Twenty-two percent of conven-
tionally staged node-negative patients were considered
to have upstaged tumors because micrometastatic tumor
deposits were detected in the sentinel node(s) by fur-
ther pathologic scrutiny beyond standard H&E evalu-
ation. Of the remaining patients with nodal involvement,
29 had pN1 disease (> 2-mm macroscopic foci of tumor
detected by H&E). Seven patients had small tumor de-
posits within the sentinel lymph nodes (≤ 2-mm foci of
malignant-appearing cells detected by H&E and/or IHC;
pN1mi[sn], n = 3; pNO[i+] [sn], n = 4). 25 One American
Joint Committee on Cancer stage T1 tumor had nodal
metastasis while 53% of T2, 70% of T3, and 33% of T4 lesions were associated with nodal metastases.

LYMPHATIC MAPPING OF THE COLONIC MESENTERY AND MESORECTUM

Surgical specimens contained an average of 18 lymph nodes, which allowed for accurate conventional staging. To assist with more accurate lymphatic staging, an average of 2.2 lymph nodes per specimen were identified as sentinel nodes by the surgeon or pathologist. These sentinel lymph nodes were most often blue after uptake of isosulfan blue from the afferent lymphatics. Sentinel nodes were identified in all patients. There were 2 false-negative sentinel nodes identified in this study population. The overall accuracy rate of the sentinel node to predict the status of the remaining nodal basin was 97%; the false-negative rate was 5.3%. No change in the extent of operation occurred in this sample set. There were no adverse reactions to the in vivo isosulfan blue injections.

EXPRESSION OF mp53 AND TSP-1 IN THE PRIMARY TUMOR

The mp53 protein was easily detected primarily in the nucleus of tumor cells. In contrast, TSP-1 protein was found primarily in the cytoplasmic compartment (Figure 1).

Overall, 54% of tumors expressed mp53. Acquisition of mp53 was significantly associated with a decreased mean TSP-1 OD value. Tumors without mp53 expression had a mean TSP-1 OD value of 51.3 vs 31.8 in tumors with an elevated mp53 concentration (P < .03) (Figure 2). Many factors can lead to the down-regulation of TSP-1; because of these factors and the limitations of the detection methods, some variation of TSP-1 OD values in patients with and without mp53 was expected.

Patients with a truly negative sentinel node(s) (pN0[i−][sn]) had significantly higher TSP-1 OD values compared with those with nodal metastases; 71.4% of node-negative patients had a TSP-1 OD value of >30 (normal) while 41.4% of the node-positive patients had a similar TSP-1 OD value (P < .047) (Figure 2). Additionally, TSP-1 OD values in patients with upstaged tumors (ie, pN1mi[sn] or pN0[i−][sn]) were similar to the TSP-1 OD values of patients with N1 tumors (28.3 vs 30.7; P = .84) and much lower than patients with negative sentinel node(s) (pN0[i−][sn]) and H&E-negative nonsentinel node(s) (28.3 vs 57.7; P < .04) (Figure 3). All patients whose tumors were upstaged by serial sectioning of the sentinel node(s) (H&E negative/IHC positive) had primary tumors that expressed mp53, while only 40% of node-negative tumors expressed mp53 (P < .003). All patients with upstaged tumors had T3 tumors with moderate to poorly differentiated features; other commonly reported histopathologic factors, including lymphovascular invasion, had no correlation in this small subset of patients (data not shown).

COMMENT

Over the past decade, numerous studies have investigated the value of angiogenesis markers in carcinoma. In some organ systems (eg, prostate and melanoma), these markers appear to have prognostic significance. We investigated the role of 2 angiogenesis markers (ie, mp53 and TSP-1) in patients who presented with resectable CRC, where recent publications suggest novel treatments supplementing or restoring these markers may have a therapeutic impact. Our study is unique in that lymphatic mapping was used to accurately stage lymphatic micrometastasis of the sentinel node(s) in addition to conventional (H&E) examination of a large number of non-sentinel nodes (average of 18 lymph nodes per CRC specimen). The sentinel lymph node mapping technique revealed approximately 22% of patients had lymphatic micrometastasis not detected by conventional techniques. Our data revealed an association between mp53, decreased expression of TSP-1, and lymphatic metastases in CRC.

In the tumor microenvironment, multiple proteins, including p53 and TSP-1, have a tumor-suppressor effect. Mutations of the p53 gene are among the most frequent genetic changes in human cancer; the subsequent lack of downstream events results in a disruption of the angiostatic and angiogenic factors promoting tumor progression. The tumor-suppressor role of p53 is further established by studies that show when p53 function is established by studies that show when p53 function is restored, tumor development slows.9 Thrombospondin 1 and other thrombospondins (eg, TSP-2), also present in this environment, can have tumor-suppressor activity that limits disease progression by inhibiting angiogenesis.11,27 Thus, novel therapeutic interventions designed to increase TSP levels in the tumor tissue reportedly can limit tumor growth.28-29 Our data suggest that decreased expression of TSP-1 is a key step in creating a
proangiogenic environment; once TSP-1 expression is decreased, there is a shift toward a higher frequency of lymphatic metastases. Interestingly, the volume of lymphatic metastases did not correlate to the TSP-1 expression because patients with micrometastases (pN0(i+)) had similar decreased TSP-1 expression compared with conventionally staged node-positive patients with macrometastases (pN1=metastasis measuring >2 mm). This finding suggests micrometastasis is an early histologic finding of an environment that has undergone a fundamental shift, a shift favoring disease progression. One must recognize that decreased mean TSP-1 expression is certainly not the sole change within the tumor microenvironment allowing for lymphatic metastasis; however, it does appear to be important in a subset of patients. These and other functional markers (eg, TSP-2) together with their corresponding lymphatic metastasis may be further studied to understand the primary tumor–lymphatic microenvironment relationship. Clinically, this understanding may lead to the ability to better guide adjuvant and novel therapies in select subsets of patients with stage II colon cancer.

At present, determining the potential benefit of adjuvant chemotherapy for an individual patient is a complex decision and relies heavily on traditional TNM staging parameters in addition to clinical and pathologic factors. Accurate staging is pivotal when attempting to predict the biology of CRC because chemotherapy clearly improves the outcome of patients with stage III CRC. The risk-benefit ratio of adjuvant therapy is less certain for patients with stage II colon cancer, even though observation after curative surgery is associated with a recurrence rate as high as 30%.

In 2004, the American Society of Clinical Oncology recommendations regarding adjuvant chemotherapy stated there was no direct evidence from randomized trials to support the routine use of adjuvant chemotherapy in patients with stage II colon carcinoma. However, the significant impact of chemotheraphy on stage III disease supports its consideration for use in high-risk patients with stage II disease. Therefore, establishing prognostic factors to identify a high-risk subset of patients with stage II disease has been an important focus because it could change adjuvant therapy. Since that time, Gray and colleagues in the QUASAR Collaborative Group reported a statistically significant survival advantage for adjuvant treatment of stage II colon cancer. To our knowledge, this was the first randomized trial to demonstrate a survival benefit in this group (1%-5%). Although chemotherapy is likely to help some node-negative patients, the subsets of patients who truly benefit still need to be identified. Currently, treating the high-risk and young patients usually outweighs the inconvenience, cost, and toxic effects of such treatment. Its findings and the increasing efficacy of newer chemotherapeutic regimens have increased the pressure to treat more patients with stage II disease. As the trend shifts toward treating the majority of patients with stage II disease and away from
observation, there will likely be a significant amount of overtreatment.

Considering the need to identify subsets of patients with stage II colon cancer to help guide adjuvant therapy, a particularly interesting observation of our study was that patients without macrometastases or micrometastases in sentinel and nonsentinel nodes maintained a high (protective) TSP-1 expression. The average TSP-1 OD value for pN0(i−)(sn) patients was 57.7, whereas patients with any degree of nodal metastases had an average TSP-1 value of 30.1 (P < .001). Hence, the clinical value of angiogenesis markers may be in using them, as well as other clinical and pathologic markers, in conjunction with step sectioning of the sentinel lymph nodes to identify a low-risk group of patients (ie, pN0[i−][sn] with high TSP-1 expression) who may safely avoid adjuvant chemotherapy. As observed in other diseases, there now appears to be a new generation of “node-negative” (ie, sentinel node–negative [pN0(i−)(sn)]) patients with CRC with significantly improved outcomes when compared with historic controls.19,34-36

In summary, we feel these data suggest TSP is involved in the acquisition of a phenotype of CRC that favors lymphatic metastasis. Further studies are warranted to investigate the prognostic value of maintaining high/normal levels of this and other functional angiogenesis markers combined with accurate lymph node analysis; these studies will investigate the relationship between TSP expression and the mutations associated with them, disease progression, and survival.

Accepted for Publication: February 24, 2007.

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Author Contributions: Study concept and design: Iddings, Saha, and Bilchik. Acquisition of data: Iddings, Gelwal, Parker, and Saha. Analysis and interpretation of data: Iddings, Koda, Gelwal, Parker, and Saha. Drafting of the manuscript: Iddings and Parker. Critical revision of the manuscript for important intellectual content: Iddings, Koda, Gewal, Parker, Saha, and Bilchik. Statistical analysis: Parker. Administrative, technical, and material support: Koda and Parker. Study supervision: Bilchik.

Financial Disclosure: Dr Parker is an employee of OncoCote Inc.

Funding/Sponsor: The research was performed pursuant to agreement under Research Project Grant CA90484 with the National Institutes of Health, Department of Health and Human Services and was supported by funding from Mrs Ruth Weil (Los Angeles, California), the Rod Fasone Memorial Cancer Fund (Indianapolis, Indiana), the Henry L. Guenther Foundation (Los Angeles), the Rod Fasone Memorial Cancer Fund (Indianapolis, Indiana), the Harold J. McAlister Charitable Foundation (Los Angeles), the John Wayne Cancer Institute Auxiliary (Santa Monica, California), The Wrath Family Foundation (Los Alamos, California), Patricia C. Brown Foundation (Costa Mesa, California), the Family of Robert Novick (Los Angeles), and The Samuel Foundation (Corona del Mar, California).

Previous Presentation: This paper was presented at the 78th Annual Meeting of the Pacific Coast Surgical Association; February 18, 2007; Kohala Coast, Hawaii; and is published after peer review and revision. The discussions that follow this article are based on the originally submitted manuscript and not the revised manuscript.

REFERENCES

Oscar Joe Hines, MD, Los Angeles, California: Dr. Bilchik, your group has pioneered the area of sentinel lymph node biopsy in colon cancer, and, although the utility of this procedure has yet to be determined, this project and the collection of specimens allow you to begin to answer several other interesting questions. Today you presented data suggesting that in early colorectal cancer, primary tumor thrombospondin and mutant p53 correlate with nodal disease. Specifically, when the primary tumor expressed mutant p53, decreased thrombospondin was identified and this correlated with the likelihood of nodal metastasis. In fact, in patients with standard H&E-negative nodes but positive nodes by immunohistochemistry, intermediate levels of thrombospondin were found in comparison to patients with H&E-positive nodes.

Your group hopes to identify a subgroup of stage II colorectal patients that would benefit from adjuvant treatment. As many as 30% of patients with stage II disease will go on to progress to advanced cancer. You conclude that tumors with mutant p53 have diminished thrombospondin and increasing the chance of nodal disease. With that background, I have 5 questions.

For a tumor to continue to grow, it must recruit additional blood supply. This process includes several key factors and events that ultimately trigger an angiogenic switch to a proangiogenic environment. Thrombospondin is a well-known angiostatic protein that decreases with tumor growth, allowing for proangiogenic factors like VEGF [vascular endothelial growth factor], PDGF [platelet-derived growth factor], FGF [fibroblast growth factor], and ELR [glutamic acid–leucine-arginine]-positive chemokines, including IL-8, ENA [epithelial-derived neutrophil attractant]-78, and GRO [growth-related oncoprotein]-alpha, to create a proangiogenic tumor. Lymphangiogenesis appears to be a separate process from angiogenesis and involves other factors like VEGF-C and its corresponding receptor VEGFR3. Thrombospondin has not specifically been implemented in lymphangiogenesis.

Although the process of nodal metastasis is a complex one that involves several factors and events outside of lymphangiogenesis, why have you chosen to examine thrombospondin in relation to nodal disease? Have you examined other factors that may be specifically related to lymphangiogenesis, and more broadly angiogenesis, that I have listed?

Your data shows that thrombospondin levels were commonly down-regulated in tumors with p53 mutations. However, some patients with mutant p33 had equivalent levels of thrombospondin to those that did not express mutant p53. How do you explain this? To that end, what is the mechanistic relationship between p53 and thrombospondin, or are you simply observing an epiphenomenon?

The focus of your paper has been nodal status, but you have examined the primary tumor. Have you had the chance to examine the nodes for thrombospondin and p53 status? If not, what would you hypothesize the findings would be?

You hope this data might be able to predict those patients who will progress. Do you have any clinical information about the disease progression in this group of patients and how has that correlated with the findings of this study?

Lastly, with regard to sentinel nodes in colorectal cancer, this concept has been met with mixed reviews. Can you give us an update on what direction you think this concept is heading in colorectal cancer and do you think it will impact patient outcome?

Again, thank you for the opportunity to discuss your paper and congratulations.

Dr. Bilchik: Although colon cancer is the second most common cause of cancer-related death in the United States, recent statistics have shown a dramatic improvement in survival. This is likely related to more effective screening, but it is also the result of novel systemic and biological agents that are effective in up to 80% of patients with advanced disease. The paradigm, however, is shifting to treat patients with early colon cancer despite little survival benefit and enormous expense. The trials that have led to approval of these agents have not focused on the efficacy of surgery or pathologic reviews, and surgeons have typically not been involved in the designs of these trials.

Our study and others demonstrate that up to 23% of nodal metastases are missed by conventional pathological techniques. The prognostic value of these micrometastases has been the subject of much debate, and some would argue that profiling of the primary tumor is likely to be more important. I believe that it is likely to be a combination of both.

The development of lymphatic metastasis is indeed complex and likely occurs when there is an imbalance of angiogenesis and angiostatic factors. Of the numerous angiogenesis factors involved, thrombospondin was selected because it is a potent inhibitor of angiogenesis within the tumor microenvironment and has a potent direct effect on endothelial cells by inhibiting migration, thereby inducing apoptosis. Because of this, several therapeutic approaches, including cell-based gene therapy and recombinant proteins that increase thrombospondin, are being developed.

Several oncogenes and tumor-suppressor genes including p53 have been shown to regulate thrombospondin, but the details of the signaling pathway have only been determined for ras. In various genetic models, more rapid tumor progression is observed in p53-deficient mice in the absence of thrombospondin. The direct interaction of p53 and thrombospondin, however, has not been clearly demonstrated.

You asked why thrombospondin levels do not always correlate with p53 mutations. This is likely attributed to the ac-
Ko, you recently put together a very nice study with multiple risk factors and tumor factors, etc., and all to derive best therapy for colon cancer. Does this appeal to you, the markers that Dr Bilchik and Dr Iddings have talked about, as a potential part of a formula that you would work into managing colorectal carcinoma?

Clifford Y. Ko, MD, Los Angeles: What Dr Goodnight is referring to is a study we performed at UCLA [University of California, Los Angeles] and RAND. It was an appropriateness panel where we identified performance measures, or quality indicators, for colorectal cancer care. We performed thorough systematic literature reviews and assembled an expert panel to help determine what is good and what is not good. An issue with developing any type of quality measure is that we all want the measure to be evidence based, but as we all know, there is not enough evidence in the literature for everything we do. And the same is true in colorectal cancer. In our study, this node issue was discussed at length. The panelists agreed that the node count has to be a certain level because everyone agreed that the more nodes you get the better sample you get and the better you can determine the stage correctly. We used 12 nodes as the cutoff. The group agreed that if 12 nodes were not identified, the pathologist would be asked to reinspect the specimen. If 12 nodes were still not identified, then the patient would be referred for a discussion to a medical oncologist. We did start to address the issues of micrometastasis; however, as I mentioned earlier, the problem was that there wasn’t enough appropriate literature to say anything definitive. We didn’t have enough available information in the literature when we performed our study. It seems that now, however, increasing amounts of evidence seems to point out that if one can elucidate more and better information with an increasingly sensitive and specific test, it seems logical that such a test should be used, and Dr Bilchik is one of the people leading the way in this regard.

Stanley P. L. Leong, MD, San Francisco, California: I think that once we are to reliably achieve 12 nodes in our examinations, then we can start asking our pathologists to perform appropriate examinations to look for micromets. So we should have a short-term plan of at least achieving some number, some floor number, which we are having trouble doing, but then have a longer-term plan of possibly examining nodes for micromets as a next step.

Dr Leong: I agree with that. Just to add quickly onto this, when you take out those 12 lymph nodes, you make one section only for each of these 12 lymph nodes, but when you get that sentinel lymph node out, you cut this lymph node in multiple sections and therefore the yield of finding micrometastasis is much higher than a single section for each one of these 12 lymph nodes.

Dr Goodnight: This panel is known for its demure reticence, but we will try to get them to talk.

Jan H. Wong, MD, Honolulu, Hawaii: I just have a quick comment about the micrometastatic disease issue. It is somewhat controversial in many people’s minds and that’s because the literature really is quite variable on how this story plays out. What we should remember is that in the literature the significance or nonsignificance of the biologic impact or prognostic impact of micrometastatic disease is so variable. The data that this is based on does not meet what we would consider a sufficient surgical removal of nodes or optimal pathologic nodal analysis. The mean number of nodes in almost all of those studies is substantially less than 10 so the actual validity of those analyses comes into question.

Dr Goodnight: Drs Iddings and Bilchik, it looks like you have security in employment. There is enthusiasm here for sentinel lymph node testing.

Financial Disclosure: Dr Leong received an educational grant from and is on the speakers’ bureau for Schering-Plough Oncology and received educational grants from Neoprobe Inc and Ethicon of Johnson & Johnson.