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Do Primary Neuroendocrine Tumors and Metastasis Have the Same Characteristics?

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Background. **Only one tumor site is usually biopsied to determine the histologic features of that patient's entire tumor burden. We hypothesized that there are significant histologic and functional differences in primary neuroendocrine tumors (NETS) and their nodal or organ metastases. We also hypothesized that limited tumor sampling could lead to erroneous assumptions about the tumor's histologic characteristics and clinical behavior.**

Materials and Methods. **Thirteen patients with metastatic well differentiated midgut NETS underwent simultaneous removal of their primary tumor, nodal metastasis, and organ metastasis. Each tumor site was stained quantitatively for Ki-67, chromogranin A (CGA), synaptophysin, CD31, and Factor VIII. Samples were also evaluated with *in vitro* tumor angiogenesis and drug chemoresistance assays.**

Results. **Ki-67 staining was nearly identical at all sites tested. Quantitative stains for CGA, synaptophysin, cluster of differentiation 31 (CD31), and Factor VIII varied considerably among the patient's three tissue site samples. Only 6% of the tissue samples tested against a battery of chemotherapeutic agents exhibited susceptibility to a single drug at all three tumor sites. In contrast, several antiangiogenic agents exhibited uniform effectiveness across all three tissue sites in multiple patients.**

Conclusions. **Sampling only one NET tumor site may lead to erroneous assumptions about the tumor's histologic features and functional behavior. Evaluation of primary tumors and their nodal and organ metastasis may be necessary to optimize clinical decision making.**

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INTRODUCTION

Current treatment of metastatic neuroendocrine tumors (NETS) involves a multifaceted approach. Surgical cytoreduction is the mainstay of treatment in order to control tumor burden in patients whose tumor is feasibly resectable. In addition to surgery, a multitude of therapeutic options exist and are commonly used during the lifetime to control disease progression. These include somatostatin analog therapy, chemoembolization, radioembolization, intra-arterial and systemic chemotherapy, peptide receptor radiotherapy (PRRT) and antiangiogenic therapies, depending on the location and extent of tumor burden [1].

Many of these therapies are chosen based on the tumor's histologic or functional biologic characteristics. Physicians commonly biopsy one tumor site and rely on that single sample to accurately represent the entire spectrum of the patient's tumor burden. Some studies suggest that there is a vast amount of diversity among tumors of different locations within the same individual and that sampling multiple tumor sites is critical to obtain optimal information for treatment planning.

In 1986, Von Hoff *et al.* measured *in vitro* drug sensitivities in 99 pairs of tumor samples from the same patient [2]. They compared the drug sensitivity of multiple sections from the same primary tumor and also the drug sensitivity of metastasis *versus* that of the primary tumor. This study showed poor correlation between the drug sensitivity profiles of primary *versus* metastatic tumors ($P = 0.84$) and between metastasis *versus* metastasis ($P = 0.39$) from the same patient. In

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contrast, multiple sites within a single primary tumor showed excellent correlation ($P = 0.002$). These authors concluded that evaluating drug sensitivity in multiple samples from the primary tumor is not necessary; however, they recommended sampling and testing as many sites of metastatic tumor as practical.

This study suggests that there may be significant histologic and functional biologic differences in neuroendocrine primary tumors and their nodal or organ metastases.

Neuroendocrine tumors have shown minimal response to cytotoxic therapies, with the exception of high grade pancreatic and gastric tumors. When multiple chemotherapeutics, most commonly 5-fluofouracil, doxorubicin, or streptozocin are utilized, the response rate is typically around 30% for all NETS [3]. A recent trial, however, conducted by the Eastern Cooperative Oncology Group, found no clear benefit in patients receiving cytotoxic therapy with small bowel primary tumors [4]. The low response rate can be contributed to the low proliferation index characteristically exhibited by these tumors. Atypical carcinoids, as well as foregut tumors, typically have a better response rate.

In order to better understand underlying tumor characteristics and their relationship to efficacious treatment, we prospectively studied the neuroendocrine tumors of patients who had cytoreductive surgery during which primary tumor, lymph node, and organ metastases were resected. These samples were compared according to results of histologic stains assessing commonly compared characteristics, as well as a preselected battery of chemotherapeutics and antiangiogenics to determine their resistance profiles.

We hypothesized that sampling only one tumor site will not provide a complete representation of the clinical condition. We also hypothesized that limited tumor sampling can lead to erroneous assumptions about the tumor's histologic characteristics and clinical behavior.

MATERIALS AND METHODS

Tissue Acquisition

This study was approved by the Louisiana State University Health and Sciences Center (LSUHSC), New Orleans and Ochsner Medical Center Institutional Review Boards, and all patients signed an informed consent. We collected small bowel primary neuroendocrine tumor, mesenteric nodal metastasis, and liver metastasis samples from 13 NET patients who underwent cytoreductive surgery. The surgeon identified suspected malignant tissue and divided each specimen for processing while in the operating room. In an effort to ensure maximum consistency among testing procedures, these samples were collected during the same operation, stained by the same technician, and evaluated by the same pathologist at the same time.

Histology

Each of the samples was stained with the MIB-1 antibody to quantitate cellular proliferation [3]. This stain identifies the number of actively dividing cells in the tumor sample, which is represented as the Ki-67. The samples were divided into three categories based on the percent of MIB-1 positive cells (2% or less, 3%–20%, and >20%). These categories correspond to the older terminology of typical, intermediate, and atypical carcinoid, respectively. Newer grading classification schemes from NANETS (North American Neuroendocrine Tumor Society) would deem these categories as G1, G2, and G3, respectively [4].

Quantitative chromogranin A (CGA) and synaptophysin stains are used in our practice to evaluate the degree of tumor differentiation [5]. The samples were graded by the percent (0%–100%) of cells that stained with a specific intensity (+3). Samples were classified as having either high (100%–90%), intermediate (89%–71%), or low staining (0%–70%) depending on the percent of cells with +3 staining for the respective marker. These intensities are somewhat subjective and to eliminate potential bias the same pathologist evaluated all three specimens from an individual at the same sitting to ensure maximal concordance of scores.

The angiogenic index for each tumor specimen was determined by both cluster of differentiation 31 (CD31) and Factor VIII stains. Results of CD31 and Factor VIII stains were reported as the number of vessels that stained positively per high powered field. Ten "hotspots" per sample were averaged to give a final result. The average number of vessels per high powered field were classified as low (0–15), medium (16–30), or high (>30). Again, the same pathologist interpreted each tissue site during the same session to minimize the errors induced by using multiple readers (pathologists) or one person reading the specimen slides from different sites at different sessions.

Angiogenesis Assay and Evaluation

In vitro evaluation of the tumor's ability to generate an angiogenic response was performed using a proprietary human tumor angiogenesis assay developed in our LSUHSC laboratory [6]. Tissue embedded fibrin clots were prepared as previously outlined [7–9].

Briefly, tumor specimens were cross-blade minced to generate fragments (≤1 mm) representative of the entire surgical specimen. Fragments were randomly distributed to multiwell plates preloaded with thrombin (0.05 IU/mL; Sigma Chemical Company, St. Louis, MO). The fragments were covered with a solution of fibrin (0.3% Sigma), ε-aminocaproic acid (0.5%; Sigma) in medium 199 (Mediatech, Herndon, VA), and supplemented with an antimicrobial solution (100 Units/mL sodium penicillin G, 100 μg/mL streptomycin sulfate, 25 μg amphotericin B; Gibco/Invitrogen, Grand Island, NY).

The tumor-containing clot was then supplemented with growth medium [(medium 199, antimicrobial solution, and 20% fetal bovine serum (Gibco/Invitrogen)] or growth medium containing one of the drugs. Preferential allocation of fragments to one treatment was minimized since the entire tumor specimen was minced prior to distribution and the distribution into the assay plates was random.

Thirty (30) fragments (wells) were treated with each compound for each specimen evaluated. Total well volume was 200 μL. Nutrient or drug treatments were added on d 0 and replenished on d 7. The cultures were maintained for 14 d at 37°C 6% CO_2.

Angiogenic evaluation was performed using an inverted phase-contrast microscope. Tumor fragments were graded using two criteria: initiation of angiogenic response and the degree of subsequent neovessel development. Initiation of an angiogenic response was defined as the development of three or more sprouts around the periphery of the fragment, visible at ×10 magnification. Percent initiation is a ratio calculated from the number of fragments (wells) that developed an angiogenic response divided by the total number of fragments prepared (×100).

For the angiogenic index (AI), each tumor fragment was visually divided into four quadrants and each quadrant was rated on a 0 to 4 scale for the amount (length, density, and fragment surface) of angiogenic growth. A total score (sum of the four quadrants) of 0 to 16 was created for each fragment. The AI for each treatment group was then averaged in one of two manners: (1) average of fragments that had an AI of 1 or greater (AI growth), and (2) average of all fragments

prepared (AI overall angiogenic response). The first calculation is considered a measure of neovessel growth. The second calculation, because it averages all fragments regardless of whether or not an angiogenic response was observed, is considered an overall angiogenic response.

Previous experience using this scoring system (AI score 0-16) showed an excellent correlation among multiple trained observers and also between unbiased observer scores and more objective ratings, such as vessel length (mm) or a total vessel surface area (mm^2) as determined by digital image analysis [8].

Chemoresistance Assay

Tumor samples were also tested for resistance to the cytotoxic effects of up to 9 preselected chemotherapeutic agents. These agents were chosen based on their potential use in the clinical setting. The drug resistance assay begins with viable tumor cells suspended in an agarose media with a single antineoplastic agent added [10]. The concentrations of reagent were higher than clinically achievable doses. The wells incubated for 72 h, after which 5 *u*Ci of titrated thymidine was added to each well. After an additional 72 h of incubation, cells were lysed and contents harvested for determination of amount of proliferation. Proliferation was measured by titrated thymidine-DNA incorporation (recorded as counts per minute) and results were reported as percent cell growth inhibition as compared to media-exposed controls. These drug chemoresistance assays were performed at Genzyme (Los Angeles, CA). A total of 82 chemoresistance assays were performed, with each assay repeated across the set of three tissues from a single patient. Up to nine chemotherapy agents were tested in each tumor specimen depending on the amount of material available for testing.

RESULTS

Histology

Three tumor samples from three different locations were resected from a total of thirteen patients with neuroendocrine tumors. The tissues were classified by their Ki-67, CGA, synaptophysin, CD31, and factor VIII tumor staining.

Tumor samples stained for Ki-67 were classified by the percentage of actively dividing cells (Table 1). In 10/13 (77%) patients, all three tumor specimens were categorized as typical or Grade 1 (G1) meaning their Ki-67 values were less than 2%. The remaining three patients had a single site demonstrating a higher Ki-67, of which all were classified as intermediate (G2, 3-20% proliferation). Of these three intermediate specimens, one was a primary tumor (Ki-67 of 5%) and two were liver metastasis (Ki-67 of 3% and 5%). There were no tumor samples in this study classified as G3 or "atypical" with Ki-67 values greater than 20%. The maximum intra-patient disparity between two locations was 5% (0% and 5%).

CGA and synaptophysin tissue stains are commonly used as markers of cellular differentiation. CGA and synaptophysin staining was graded as Grade I, II, or III according to the percent of cells that had intense (+3) staining for CGA or synaptophysin (Table 1). Of the CGA stains, 61.5% (8/13) of patients had uniform results in all three specimens. Of the remaining 38.5% (5/13) discordant samples, 4/5 had a single site that varied (one primary, one lymph node, and two liver samples) and 1/5 specimens had results that were disparate in all three locations. The maximum intra-patient disparity between two locations was 90% (100% *versus* 10%).

Synaptophysin stains demonstrated uniform results in only 46% (6/13) of the patients. More than half of

TABLE 1

Comparison of the Grade of Cells Staining Positively for Ki-67, CGA, Synaptophysin, CD-31, and Factor VIII in Primary Tumor *versus* Lymph Node *versus* Organ Metastases

Patient	Location	Ki-67*	CGA†	SYNP†	CD31‡	FVIII‡
1	1°	I	III	III	III	III
	LV	I	III	III	III	III
	LN	I	III	III	III	II
2	1°	I	I	III	I	I
	LV	I	I	III	I	I
	LN	I	I	III	I	I
3	1°	I	I	III	III	III
	LV	I	I	III	III	II
	LN	I	I	III	III	III
4	1°	I	II	I	I	I
	LV	I	III	I	I	I
	LN	I	III	I	I	I
5	1°	I	I	I	I	I
	LV	I	I	III	I	I
	LN	I	III	II	I	I
6	1°	I	III	I	I	I
	LV	II	I	I	I	I
	LN	I	III	III	I	I
7	1°	I	I	I	I	I
	LV	II	I	I	I	I
	LN	I	I	I	I	I
8	1°	I	I	III	II	I
	LV	I	I	III	II	II
	LN	I	I	I	II	II
9	1°	I	III	III	II	I
	LV	I	I	III	III	III
	LN	I	II	II	III	III
10	1°	I	I	III	I	I
	LV	I	I	I	II	I
	LN	I	I	II	I	I
11	1°	II	I	I	I	I
	LV	I	III	I	I	I
	LN	I	I	I	I	I
12	1°	I	III	II	III	II
	LV	I	III	III	III	II
	LN	I	III	II	III	III
13	1°	I	III	III	I	I
	LV	I	III	I	II	II
	LN	I	III	I	II	I

1° = primary tumor site; LN = lymph note.

*Ki-67 graded percent of cells actively dividing with I: 0%–2%, II: 3%–20%, and III: >20%.

†CGA and SYNP graded according to the percent of cells with 3+ intensity staining with I: 100%–90%, II: 89%–70%, and III: <70%.

‡CD31 and FVIII graded as the number of vessels per high powered field over an average of 10 fields with I: 0–15 *vpf*, II: 15–30 *vpf* and III: >31 *vpf*.

the tumor trios (7/13) demonstrated heterogeneous staining between tumor locations. Five of 13 (5/13) sets differed by a single site, with the disparate sites located in one primary, one liver, and three lymph nodes. The remaining 2/13 patient specimens exhibited different staining intensities for synaptophysin at all three sites. The maximum intra-patient disparity between two locations was 95% (100% and 5%).

CD31 and factor VIII are widely used to quantify the number of blood vessels per high powered field (Table 1). The number of vessels per high powered field were categorized uniformly in all three tissue specimens in 10/13 (77%) of the patients by CD31 stains. The remaining three patients exhibited a single site categorized as either more or less angiogenic. Factor VIII stains were uniform in 7/13 (54%) patients with the remaining six patients exhibiting single site variability. In 10 patients, at least five of the six samples were uniformly categorized, which demonstrates the level of agreement between the two tests.

Angiogenesis Assay

The *in vitro* functional angiogenesis assay results suggested that there are differences in a tumor's angiogenic potential when the tumors ability to generate an angiogenic response was compared among three distinct tissue sites in the same patient (Table 2).

Tumor fragments were graded by the percent of wells that began to grow (angiogenic initiation). The percent initiation was calculated using the formula: [the number of wells that developed neovessels/number of wells plated] $\times 100$. Angiogenic initiation was highest in the lymph nodes with an average of $78\% \pm 22\%$ of the wells exhibiting neovessel development. Liver metastases showed the least initiation with only $66\% \pm 27\%$ of the wells developing an angiogenic response. Comparing the three tumor sites among patients, 7/14 (one patient had the highest score in two locations) or 50% of the patients had the greatest amount angiogenic initiation in the lymph nodes, compared with 3/14 (21%) located in the primary tumor and 4/13 (31%) located in the liver metastases.

Additionally, tumor fragments were graded for the amount of growth, or angiogenic growth. The percent growth was calculated as the mean angiogenic score of all wells that developed neovessels. The lymph nodes exhibited the highest percent growth in 8/14 (57%) samples. This was followed by the primary tumor in 4/14, and finally the liver in only 2/14 samples.

The overall angiogenic potential in tumor samples was assessed *via* the angiogenic index (AI). The AI is calculated by grading each quadrant of well on a scale of 0–4 with a maximum score of 16. The AI was highest in lymph nodes for 8/14 (57%) samples, in the primary for 5/14 (36%), and in the liver for 2/14 (14%).

TABLE 2

Tissue Samples with the Highest Overall Angiogenic Effect

Tissue	Angiogenic initiation	Angiogenic growth	Overall effect
Primary	3*	4	4*
Liver metastasis	4	2	2
Lymph node	7*	7	8*

Number of tumor samples with the highest score in each category per patient. The overall effect shows 8/14 had the most angiogenesis in the lymph nodes.

*The value for the primary and lymph node was identical for one patient.

Anti-Angiogenic Testing

Fifteen different antiangiogenic agents were tested against tissue from the three tumor sites from13 patients to yield a total of 147 unique drug–tumor sets (Table 3). A positive response was defined as greater than 50% decrease in angiogenesis (compared with untreated control wells) after treatment. Overall 62/147 (42.2%) of tissue sets were uniformly responsive to treatment across all three tumor sites. Conversely, 43 (29.3%) sets responded poorly to therapy in all tumor locations. Of the remaining sets, 24 (16.3%) responded in two locations and 18 (12.2%) showed response to therapy at only one location. The primary tumor, lymph node, and metastases specimens were similarly sensitive to the antiangiogenic agents at 37%, 34%, and 30% of samples responding, respectively.

In vitro Chemoresistance Testing

In vitro chemoresistance assays were performed on patients' ($n = 13$) samples from the three tumor locations. Each tumor specimen was tested against up to nine chemotherapies (Table 4). A total of 82 unique drug–tumor sets were generated. Five sets (6.1%) were uniformly sensitive (exhibited low resistance) at all three locations. In contrast, 30 sets (36.6%) were resistant to the chemotherapeutic tested at all three locations. Of the remaining samples, 26 (31.7%) were sensitive to a specific therapy in only one location and 21 (25.6%) were sensitive in two of the three locations.

DISCUSSION

Von Hoff *et al.* have shown that that multiple tissue samples from a single primary tumor respond in a similar fashion (chemosensitivity) when the tumors are exposed to a battery of chemotherapeutic agents [2].

TABLE 3

Agents that Decrease Angiogenesis by 50% or Greater Across Three Specimens: Primary Tumor, Lymph Node, and Organ Metastases

		Decreased angiogenesis by 50% or more							
Drug	*n* Tested	3 out of 3 tissues		2 out of 3 tissues		1 out of 3 tissues		0 out of 3 tissues	
Gallic acid	13	13	100%	0	0%	0	0%	0	0%
2-Methoxyestradiol	13	0	0%	1	8%	3	23%	9	69%
Avastin	8	0	0%	0	0%	3	38%	5	63%
Black raspberry	8	3	38%	3	38%	2	25%	0	0%
CP 673451	7	1	14%	2	29%	0	0%	4	57%
Patupilone	12	11	92%	1	8%	0	0%	0	0%
Gleevec	12	3	25%	0	0%	2	17%	7	58%
Interferon A	5	0	0%	0	0%	1	20%	4	80%
Vatalanib	13	7	54%	5	38%	1	8%	0	0%
Everolimus	13	1	8%	3	8%	5	38%	4	31%
Pasireotide	5	0	0%	0	0%	0	0%	5	100%
Sweet leaf tea	13	11	85%	2	15%	0	0%	0	0%
Taxol	12	5	42%	2	17%	0	0%	5	42%
Valproic acid	6	2	33%	3	50%	1	17%	0	0%
Vincristine	7	5	71%	2	29%	0	0%	0	0%
*n =	147	62	42%	24	16%	18	12%	43	29%

*A total of 147 tumor sets were tested in these angiogenic agents.

In contrast, comparison of the primary tumor's chemosensitivity profile with the chemosensitivity profile of metastatic deposits from the same patient varied widely. Finally, when these authors tested multiple metastatic sites against the same battery of antitumor agents, widespread differences in chemosensitivity to the agents were again noted.

This observation may help explain why chemotherapy regimens often fail and may help explain the rapid emergence of "drug resistant clones." The development of "resistance" is common, even when multiple chemotherapeutic agents are used in combination, but in fact "resistance" may be the lack of a homogeneous sensitivity to chemotherapy in the different tumor sites that represent the metastatic burden [11].

Few studies have attempted to assess the heterogeneity of primary- and metastatic tumor's expression of common cellular markers of proliferation, differentiation, and vascular density within the primary or metastatic tumor sites. To determine if the responses or characteristics of multiple tumor locations were homogeneous, we prospectively collected primary tumors along with metastatic specimens from the liver and lymph nodes in 13 patients with well differentiated midgut NETS undergoing extensive intra-abdominal cytoreduction. In these tumor specimens, we evaluated the proliferation index (Ki67), the histologic expression of markers commonly used to classify tumor differentiation (CGA, synaptophysin), and the histologic indicators of tumor vascularity (CD31 and Factor VIII).

TABLE 4

Incidence of Low Resistance to Chemotherapeutic Agents Across Three Specimens: Primary Tumor, Lymph Node, and Organ Metastases

		Incidence of low resistance							
Drug	*n* Tested	3 out of 3 tissues		2 out of 3 tissues		1 out of 3 tissues		0 out of 3 tissues	
Cisplatin	11	0	0%	0	0%	3	27%	8	73%
Dacarbazine	11	0	0%	5	45%	3	27%	3	27%
Doxorubicin	13	1	8%	4	31%	5	38*%	3	23%
Etoposide	11	0	0%	6	55%	3	27%	2	18%
Fluorouracil	13	2	15%	3	23%	8	62%	0	0%
Gemcitabine	1	0	0%	0	0%	1	100%	0	0%
Interferon α	8	0	0%	1	13%	1	13%	6	75%
Taxol	3	0	0%	0	0%	0	0%	3	100%
Tenozolamide	11	2	18%	2	18%	2	18%	5	45%
*n =	82	5	6.1%	21	25.6%	26	31.7%	30	36.6%

*A total of 82 tumor sets were tested in these drug resistance assays.

Ki-67 values from an individual's primary tumor, lymph node metastases, and liver metastases were identical in the three tumor sited tested in 10/13 patients. In those patients who had disparate scores from the three sites, the maximum difference between Ki-67 scores was 5%. Of the three G2 specimens, one was located in a primary tumor and two were in liver metastases specimens. Based on our small sample size, we could not extrapolate that a particular site was the most highly proliferative. Our data, however, does imply that in the average NETS patient a single tissue biopsy should effectively determine the "typical" (G1), "intermediate" (G2), or "atypical" (G3) proliferative index of the tumor.

Ki-67 is documented prognostic factor for neuroendocrine tumors and is currently used as an adjunct to tumor size to predict likelihood of metastasis [12]. Others have shown that the Ki-67 of the primary tumor, along with size of primary tumor, can predict the incidence of metastasis with statistical significance [13]. The significance of the Ki-67 proliferative index in tissue from metastasis has not been investigated and its impact of disease progression is not well understood. Our study implies that the proliferative index of primary tumors and their metastasis are similar.

We found that the tumor differentiation markers CGA and synaptophysin were uniformly expressed in about half of the patients, 61.5% (8/13) and 46.15 (6/13), respectively. There was a large intra-patient discrepancy among the degree of tumor differentiation at different tumor locations. The maximum discrepancy between tumor sites in an individual was significant, with some specimens having differences of 95%. This provides evidence that primary tumors can seed disease with varying levels of differentiation at metastatic locations.

In vitro angiogenesis testing revealed that about 50% of patients have the most active neovessel growth in the lymph nodes. This implies that some tumor sites might be more sensitive than others to the effects of antiangiogenic agents. Certainly in our clinical experience, the response of liver metastases and lymph node metastases (especially those in the root of the mesentery) vary widely when patients are treated with agents such as everolimus [14].

A battery of antiangiogenic therapies demonstrated uniform effectiveness in over 42% of the paired tumor sites that were evaluated. The blood supply to these tumor sites appears to have equivalent susceptibility to specific antiangiogenic agents. This response, however, is not uniform or universal. Sampling multiple tumor sites again would be recommended based on our results.

There was no benefit in one site compared with the others when using antiangiogenic therapies. Regardless of location, tumor samples were sensitive to antiangiogenic testing in about 35% of specimens. The utility of angiogenic testing lies in the ability of the clinician to determine the treatment specific to the location needing the most aggressive treatment.

Our results of *in vitro* chemotherapy testing reflected the experience of Von Hoff *et al.* These authors pointed out that chemosensitivity differed widely among primary tumor and their metastasis and metastasis from different sites. While we used drug resistance testing rather than drug sensitivity, the results from our study and that of Von Hoff *et al.* are the same; primary tumors and their metastasis are functionally different.

Chemotherapy is not traditionally used in well differentiated mid gut NETs. In previous studies, less than 10% of tumors responded when treated with a single therapeutic agent [15, 16].

In our study, only 6% of tumor sets showed susceptibility at all three tumor locations to a single chemotherapy, however, over 60% of samples were sensitive to at least one chemotherapeutic. It appears that the most accurate assessment of risk and the determination of the optimal therapy require sampling a variety of tumor sites. While not supported by large prospective randomized trials, the concept of individualization of treatment *via* chemoresistance assays may be a key concept in selected individuals with well differentiated mid gut carcinoid.

CONCLUSION

We have demonstrated differences in tumor stains that evaluate proliferation, differentiation, and vascular density from various tumor locations within the same patient. We feel that sampling only one NET tumor site can lead to erroneous assumptions about the tumor's differentiation, vascular density, angiogenic responsiveness, chemoresistance, and overall clinical behavior. In addition, since metastatic lesions exhibit varying responses to chemotherapy, we recommend using a combination of chemotherapeutics when tissue testing is not available or the amount of tissue available for testing is limited. The optimal chemotherapy regimen would include the most successful therapy from each sampled tumor location. Ultimately, the clinical goal is to achieve the highest level of cytotoxicity for each lesion and the widest, most effective coverage possible for the patient. Based on the results of this prospective study in patients with well differentiated midgut NETS, we believe that whenever practical it is in of best interest to the patient to obtain samples from a wide array of tumor sites. Sampling of multiple tumor sites will allow the optimal evaluation the tumor's biologic and histologic characteristics.

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