A Model for Acoustic Characterization of Intraocular Tumors

D. J. Coleman,* F. L. Lizzi,† R. H. Silverman,* L. Helson,‡ J. H. Torpey,* and M. J. Rondeau*

Human intraocular tumors and tumors derived from human tumor cell lines grown subcutaneously in the athymic nude mouse were scanned by diagnostic ultrasound. Radiofrequency scan data were converted to digital form and analyzed in the frequency domain. Characteristics of normalized power spectra were found to be significantly different among human spindle cell malignant melanomas, mixed/epithelioid malignant melanomas, metastatic carcinomas, and hemangiomas. Significant differences, as well, were found between implanted primary skin malignant melanomas and adenocarcinomas of the lung, colon, and stomach. Comparison of spectral properties of human intraocular and implanted tumors revealed that human spindle cell malignant melanomas and implanted melanomas exhibit similar characteristics. Human intraocular metastatic tumors from the lung were found to exhibit characteristics similar to those of implanted lung tumors. These results indicate that the implantation of human tumor cell lines in the nude mouse may provide a very useful model for application of diagnostic and therapeutic ultrasound modalities to human intraocular tumors. Invest Ophthalmol Vis Sci 26:545-550, 1985

The frequency-dependent reflectivity of a region of tissue subject to a focused beam of ultrasound will be related to both stochastic and deterministic characteristics of the region. Stochastic tissue factors include subresolvable scatterers of variable dimensions and geometries (ie, spherical, linear, and planar), while deterministic factors relate to discrete structural boundaries (Table 1). Mathematic modeling of the interaction of a focused beam of ultrasound with stochastic and deterministic tissue structures has provided a theoretic framework for understanding of the frequency-dependent properties of acoustic reflectivity.1

Based upon the above considerations, it is expected that human intraocular tumors of different types would show differences in their frequency-dependent reflectivity. Such differences would be dependent upon combinations of the above theoretic parameters that differ from one tumor type to another. These differences are related, in a complex manner, to differences in histology such as vascularity, melanin content, fibrosis, calcification, necrosis, and infarctive processes.

Conventional A- and B-mode ultrasonograms, while providing information relating to tumor size, location, and reflectivity, also discard valuable data contained in the original radiofrequency (rf) signal received by the transducer. Our laboratory has developed computerized techniques for the acquisition and analysis of rf data.2 We have, at the present time, accumulated a computer library of digitized rf data from nearly 1,000 ultrasound scan sessions, with typically a dozen scans acquired per session. The greater part of our library is related to intraocular tumors.

Retrospective analysis of digitized rf scan data from patients with intraocular tumors of known type has demonstrated significant differences in normalized power spectra, a measure of frequency-dependent reflectivity. Significant differences were found among spindle cell malignant melanomas, mixed/epithelioid malignant melanomas, metastatic carcinomas, and choroidal hemangiomas.3,4

While theoretic considerations lead to the expectation of spectral differences associated with differences in tumor microstructure, and observation has confirmed the existence of spectral differences in tumors of different types, the complexity of tumors as opposed to theoretic constructs makes understanding of the quantitative relationships between spectral characteristics and individual histologic factors problematic. This gap between theory and observation is exacerbated by the relative inaccessibility of intraocular tumors for immediate comparison of acoustic and
Table 1. Parameters influencing the normalized ultrasound power spectrum of tumors

<table>
<thead>
<tr>
<th>Deterministic</th>
<th>Stochastic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood vessels</td>
<td>Melanin content</td>
</tr>
<tr>
<td>Limiting or internal membranes</td>
<td>Microcalcification</td>
</tr>
<tr>
<td>Infarctive or cystic boundaries</td>
<td>Blood</td>
</tr>
<tr>
<td>within tissue</td>
<td>Microvasculature</td>
</tr>
<tr>
<td>Necrotic or fibrotic boundaries</td>
<td>Tissue microarchitecture</td>
</tr>
<tr>
<td>within tissue</td>
<td></td>
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</tbody>
</table>

parameters influencing the normalized ultrasound power spectrum of tumors. Conservative treatment of malignant melanomas has led to fewer enucleations, while metastatic carcinomas to the eye and choroidal hemangiomas generally are not enucleated. Furthermore, when histologic sections are finally available, they may not correspond to ultrasound scan planes and may have been obtained at a substantial interval subsequent to scan acquisition. As a result of this situation, only an incomplete understanding of the correlation between frequency-dependent ultrasound reflectivity and tumor histology has developed. There remains a significant gap in knowledge between theoretic models and empiric results for the frequency-dependent reflectivities of human intraocular tumors, since tumors of any given class will show a range of variation on both normalized power spectra and histology.

As an aid to fill this gap in knowledge, our laboratory has developed an animal model for ultrasonic characterization of intraocular tumors. In this model, human tumor cell lines are implanted subcutaneously in the athymic nude mouse. Tumors, when of sufficient size, are scanned with a digital ultrasound system in a manner similar to that applied during examinations of human intraocular tumors.

The model tumors have several features in common with human intraocular tumors. They are similar in size and have well-defined boundaries and a sharp acoustic impedance mismatch at their anterior borders (vitreous–retina–tumor in the eye, waterbath–skin–tumor in the mouse model).

Comparisons of normalized power spectra of implanted tumors with each other and with human intraocular tumors permits study of macrostructural and microstructural parameters associated with tumor histology that are responsible for observed spectral differences between human intraocular tumors.

The results of spectral comparisons between implanted tumors in the nude mouse and human intraocular tumors also have implications relating to the treatment of intraocular tumors by ultrasonically induced hyperthermia, which also is being investigated by our laboratory. Conversion of acoustic energy to heat is affected by tissue factors that affect the diagnostic ultrasound power spectrum. This model thus is of use in elucidating the relationship between internal tumor heating and spectral properties.

This report describes our results for spectral comparisons of four classes of human intraocular tumors and four classes of implanted tumors in the athymic nude mouse.

Materials and Methods

Cellular suspensions from human primary skin malignant melanoma (RPMI 793) and adenocarcinomas of the lung (A549), colon (AT29), and stomach (REI) were implanted subcutaneously in athymic nude mice. Implanted tumors were scanned by diagnostic ultrasound when they have grown to 5 mm or more in diameter; typically, this growth required 10–14 days following implantation.

The use of animals in this investigation adheres to the ARVO Resolution on the Use of Animals in Research.

Animal facilities used to conduct the experiments are managed centrally by the Cornell University Division of Laboratory Animal Medicine. The facility is fully accredited by AAALAC, demonstrating compliance with the NIH “Guide.” Furthermore, all physical plant construction, as well as animal maintenance, husbandry, and transportation, are in compliance with the Laboratory Animal Welfare Act (PL 89-544, PL 91-579, and PL-279), and we comply with the principles for the use of animals (NIH Manual Chapter 4206).

In addition, all use of anesthetics and tranquilizers for restraint followed guidelines as discussed in the Cornell University Medical College Research Animal Resource Center Users Guide and in consultation with center veterinarians.

Human intraocular and subcutaneous tumors in the nude mouse were scanned by an immersion technique using a focused 10 MHz transducer in pulse-echo mode. During a scan session, the rf signal received by the transducer from a gated sector approximately 7.5 mm in anterior to posterior dimension was digitized and stored on magnetic disc. Data were acquired at a sampling rate of 100 MHz along 100 adjacent scan-lines per scan; 1,024 samples were acquired for each scan line, so that each scan provided 100 Kbytes of data.

Data acquisition and analysis was performed by a DEC PDP 11/60 minicomputer with an interactive graphics system and interface subsystems described in previous reports.

Analysis of the stored data begins with conversion of stored, digitized rf data to a B-mode image that is displayed on a video monitor. Using an interactive
software package, the user, by cursor control, defines the region of interest within the displayed scan. A box of user selectable dimension describes the limits of the tissue region from which rf data is to be analyzed. Several boxes are placed, and box size may be tailored to best match the tumor dimension, so that the resultant graph is representative of the tissue. Box placement is the only part of the examination that involves subjective interaction. Effects of observer interpretation are neutralized by averaging of multiple scan planes.

The digitized rf data from each scan line segment within defined region is multiplied by a Hamming function and subjected to fast Fourier transformation to provide individual estimates of the tumor spectrum. The squared magnitudes of spectra from all scan lines in the region then are averaged to form the tumor power spectrum. When the tumor power spectrum is divided by the system calibration spectrum of the echoes from a glass plate aligned perpendicularly to the transducer at the focus, the result is a normalized tumor power spectrum. This is plotted on the video monitor as amplitude (in dB) relative to the glass plate spectrum as a function of frequency from 5 to 15 MHz, the band of useful signal-to-noise level. The linear regression equation of this plot provides values for the three parameters of the power spectrum that we have found to be collectively more significant for tumor characterization; specifically, these parameters are spectral slope (dB/MHz), intercept (dB), and statistical standard error at the intercept, or simply residual (dB). The values of these parameters for three or more scans are averaged and the average values used in all further analysis.

While individual spectral parameters may differ from one type of tumor to another, their usefulness is enhanced by a multivariate approach. Linear discriminant analysis of spectral parameters from tumors of known pathology is used to define canonical discriminant functions that are more effective for acoustic tissue characterization than a univariate approach. The values of discriminant functions for unknown tumors is the most efficacious means for comparison with our data base of tumors of known type.

Results

Figures 1 and 2 show computer-reconstructed B-mode images of an intraocular spindle cell malignant melanoma and a subcutaneous implanted malignant melanoma in the nude mouse. Associated with each image on the video monitor is the normalized ultrasound power spectrum derived from the boxed region in the central tumor area.

The spindle cell malignant melanoma shows a spectral pattern that is typical of this class of relatively homogeneous tumors, having a positive slope and low amplitude. The spectrum of the implanted malignant melanoma is quite similar to that of the intraocular spindle cell tumor.

Figures 3 and 4 show images and spectra for human intraocular metastatic carcinoma of lung origin and an implanted lung tumor in the nude mouse, respectively. The spectrum of the intraocular tumor shows a decreasing slope pattern typical of uveal metastatic carcinomas of both breast or lung origin.
The implanted lung tumor in the nude mouse has a spectrum similar to that of the corresponding intraocular tumor. Both spectra show moderately high amplitude and a negative slope, reflecting the relative heterogeneity of the tissue.

The mean spectral properties of various types of human intraocular tumors and implanted tumors in the nude mouse are summarized in Table 2. Table 2 shows a trend of decreasing slope and residual and increasing amplitude as one proceeds from the most homogeneous class of intraocular tumors (spindle cell malignant melanomas) to the most heterogeneous class (choroidal hemangiomas).

A more vivid comparison of spectral properties of intraocular and implanted tumors may be obtained from Figures 5, 6, and 7. Figure 5 illustrates the distribution of discriminant score means (plus and minus two standard errors) for human intraocular tumors. Figure 6 is the corresponding plot for implanted tumors in the nude mouse.

Figure 5 demonstrates that each of the four human intraocular tumor populations have distinct differences in their spectral properties. Figure 6 shows that the implanted malignant melanomas have spectral properties that make this a distinct population relative to the remaining three groups of implanted tumors. Of the remaining three groups, colon and stomach tumors show a clear separation. There remains a degree of overlap between lung tumors and colon and stomach tumors.

In Figure 7, the human intraocular tumor and implanted mouse tumor populations from Figures 5 and 6 are overlaid, allowing comparison of spectral properties of intraocular tumors with implants. The plot shows overlapping of distributions between uveal spindle cell malignant melanomas and subcutaneously implanted primary skin malignant melanoma, and between intraocular metastatic carcinoma of lung origin and implanted lung tumors.

Table 2. Mean slope, intercept, and intercept uncertainty of linear best-fit equation of normalized power spectra for human intraocular tumors and human tumor xenografts in the nude mouse

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>N</th>
<th>Slope (dB/MHz)</th>
<th>SE</th>
<th>Intercept (dB)</th>
<th>SE</th>
<th>Residual (dB)</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human intraocular</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spindle cell</td>
<td>24</td>
<td>0.484</td>
<td>0.062</td>
<td>-72.19</td>
<td>0.919</td>
<td>2.68</td>
<td>0.031</td>
</tr>
<tr>
<td>Mixed/epithelioid</td>
<td>20</td>
<td>0.185</td>
<td>0.052</td>
<td>-63.43</td>
<td>0.658</td>
<td>2.41</td>
<td>0.024</td>
</tr>
<tr>
<td>Mel. ca. (lung)</td>
<td>13</td>
<td>-0.196</td>
<td>0.065</td>
<td>-36.93</td>
<td>0.763</td>
<td>2.34</td>
<td>0.028</td>
</tr>
<tr>
<td>Hemangioma</td>
<td>12</td>
<td>-0.340</td>
<td>0.084</td>
<td>-51.22</td>
<td>0.866</td>
<td>2.17</td>
<td>0.039</td>
</tr>
<tr>
<td>Subcutaneously implanted</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melanoma</td>
<td>20</td>
<td>0.391</td>
<td>0.044</td>
<td>-69.77</td>
<td>0.92</td>
<td>2.63</td>
<td>0.030</td>
</tr>
<tr>
<td>Colon</td>
<td>10</td>
<td>0.033</td>
<td>0.040</td>
<td>-59.76</td>
<td>1.23</td>
<td>2.37</td>
<td>0.036</td>
</tr>
<tr>
<td>Stomach</td>
<td>14</td>
<td>0.015</td>
<td>0.070</td>
<td>-62.02</td>
<td>1.66</td>
<td>2.49</td>
<td>0.039</td>
</tr>
<tr>
<td>Lung</td>
<td>19</td>
<td>-0.119</td>
<td>0.071</td>
<td>-58.66</td>
<td>1.02</td>
<td>2.39</td>
<td>0.028</td>
</tr>
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</table>
**Discussion**

It is clear that human intraocular tumors of the four categories under consideration show distinctive properties in terms of their normalized power spectra. This observation has allowed development of techniques based upon discriminant analysis for classification, identification, and characterization of intraocular tumors.

Application of these techniques to implanted tumors in the nude mouse indicate that tumors from different sources also show distinctly different spectral properties. Comparison of the spectra of intraocular tumors with those of implanted intraocular tumors show similar spectral properties between analogous tumors, ie, spindle cell malignant melanoma with implanted malignant melanoma and metastatic carcinoma from lung with implanted lung tumors. Although choroidal and skin malignant melanomas are distinct histologic entities, these results suggest an acoustic similarity or relationship between spectra of implanted melanoma and spontaneous human choroidal melanoma. These observations are supported by reports of similar histology between implants of human tumor cell lines in the nude mouse and the original primary tumors.

Our data base, at present, does not include intraocular metastatic carcinomas with colon or stomach primaries. Ninety percent of metastatic carcinomas in our data base are from lung or breast primaries. These two tumor classes do not show statistically significant spectral differences. Other sources of metastases to the eye include the testis, esophagus, and skin malignant melanoma, but the numbers of such cases in our series are insufficient for statistical conclusions.

The fact that observable and statistically significant differences exist between classes of implanted tumors provides us with a useful model for acoustic tissue characterization. Comparisons between light, electron, and acoustic histology and the corresponding acoustic spectra can be made from one tumor type to another. Such comparisons also can be made over the life cycles of tumors of each class and following therapeutic modification as by hyperthermia or radiation, which is known to result in spectral changes in intraocular tumors.

![Fig. 5. Mean discriminant scores ± 2 SE for human intraocular spindle cell malignant melanoma (1), mixed epithelioid malignant melanoma (2), metastatic carcinoma of lung origin (3), and hemangioma (4).](image)

![Fig. 6. Mean discriminant score ± 2 SE for subcutaneous xenografts of human primary skin malignant melanoma (5), stomach (6), colon (7), and lung (8) tumors in the nude mouse.](image)

![Fig. 7. Superimposition of human and mouse discriminant distributions demonstrating overlapping spectral characteristics of human spindle cell with implanted malignant melanoma and human intraocular metastatic carcinomas of lung primary with implanted lung tumors.](image)
In particular, the spectral properties of implanted malignant melanoma and lung adenocarcinoma make these implants appropriate models for studying the effects of ultrasonically induced hyperthermia, as it may be applied to treatment of intraocular spindle cell malignant melanomas and metastatic carcinomas, respectively. While the time–temperature ratios necessary to promote tumor regression in this model will not necessarily be representative of corresponding ocular tumors, their acoustic properties make them ideal for the study of conversion of acoustic power into heating levels appropriate for tumor cell destruction.

The subcutaneous implantation of human tumor cell lines in the nude mouse provides a model for investigation of frequency-dependent acoustic reflectivity and ultrasonically induced hyperthermia. The model will provide a means for measuring conversion of ultrasonic energy into heat in a variety of tumor implants that simulate acoustic properties of specific classes of intraocular tumors. This model also may serve to elucidate correlations between tumor macroand micro-architecture and acoustic properties, thus improving the tumor differentiation or tissue typing provided by the power spectrum method of acoustic characterization.

**Key words:** intraocular tumor, acoustic characterization, power spectrum, ultrasound, athymic nude mouse

**References**


