



BIOPHYSICAL IMAGING AND COMPUTATIONAL BIOLOGY

Automated Quantification of Tumor Viability in a Rabbit Liver Tumor Model after Chemoembolization Using Infrared Imaging



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The rabbit VX2 tumor is a fast-growing carcinoma model commonly used to study new therapeutic devices, such as catheter-based therapies for patients with inoperable hepatocellular carcinoma. The evaluation of tumor viability after such locoregional therapies is essential to directing hepatocellular carcinoma management. We used infrared microspectroscopy for the automatic characterization and quantification of the VX2 liver tumor viability after drug-eluting beads transarterial chemoembolization (DEB-TACE). The protocol consisted of K-means clustering followed by principal component analysis (PCA) and linear discriminant analysis (LDA). The K-means clustering was used to classify the spectra from the infrared images of control or treated tumors and to build a database of many tissue spectra. On the basis of this reference library, the PCA-LDA analysis was used to build a predictive model to identify and quantify automatically tumor viability on unknown tissue sections. For the DEB group, the LDA model determined that the surface of tumor necrosis represented $91.6\% \pm 8.9\%$ (control group: $33.1\% \pm 19.6\%$; Mann-Whitney $P = 0.0004$) and the viable tumor $2.6\% \pm 4\%$ (control group: $62.2\% \pm 15.2\%$; Mann-Whitney $P = 0.0004$). Tissue quantification measurements correlated well with tumor necrosis ($r = 0.827$, $P < 0.0001$) and viable tumor ($r = 0.840$, $P < 0.0001$). Infrared imaging and PCA-LDA analysis could be helpful for easily assessing tumor viability. (*Am J Pathol* 2015; 185: 1877–1888; <http://dx.doi.org/10.1016/j.ajpath.2015.03.023>)

The VX2 tumor model originates from a squamous cell carcinoma that developed as a result of malignant changes in the cells of a Shope virus–induced skin papilloma in a domestic rabbit.^{1,2} This tumor model is serially transplantable in allogenic adult rabbits, easily implantable, and grows quickly in many types of organs, such as lungs,³ liver,⁴ or rectum.⁵ Therefore, the VX2 tumor blood supply is almost entirely from the hepatic artery, similar to that of humans,⁶ and rabbit hepatic arteries are large enough to permit hepatic artery catheterization.⁷ This makes the VX2 tumor a common animal model for the preclinical evaluation of new anticancer treatments^{8–11} and for new therapeutic devices, such as catheter-based therapies for patients with inoperable hepatocellular carcinoma.

The evaluation of tumor response after locoregional therapies is essential in directing management for hepatocellular carcinoma. An understanding of the various therapeutic strategies and their posttherapy imaging appearance is essential for accurately assessing treatment response. The evaluation of tumor response should include not only anatomical evaluation, such as reduction in tumor size, but also the reduction of tumor viability, the degree of induced

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necrosis,^{12–14} and the biochemical changes that occur inside tumor tissues.

During the past decade, there has been considerable interest in spectroscopy-based microscopies, which use the intrinsic optical properties of the tissues to produce histologic images without staining or exogenous markers. It was first revealed in the 1950s that neoplastic and normal tissues could be discriminated based on their infrared absorption spectra^{15,16} due to the differences in their biochemical composition. Infrared spectra directly provide a multivariate nonperturbing molecular description able to distinguish and quantify histologic constituents from tissues. Chemical concentrations are quantified by spectral absorbance properties at specific frequencies, and subtle molecular structural changes are indicated by spectral peak shifts, band shapes, and relative intensity changes.^{17,18} The coupling of the infrared spectrometer with an imaging system (microspectroscopy) further permitted to combine the measured spectrum to a spatial position on the sample and to record infrared images directly on thin tissue sections. Comprehensive studies using this technique revealed the potential for fast, stain-free, nondestructive molecular histopathologic analysis with a high spatial resolution and established the capability of infrared microspectroscopy (IRMS) to complement histopathologic tools for cancerous tissue diagnosis in different organs.^{19–25} The most recent developments in IRMS aimed at automating the procedure of tissue recognition and quantification by using statistical methods and prediction algorithms.^{26–31} These methods are based on the measurement of a large number of tissue specimens. The information gathered from this large data set is used to produce a reference spectral library of each tissue type. On the basis of this spectral library, a predictive model is elaborated and validated. Applying a model to a new tissue section generates a false colored image in which each color corresponds to a type of tissue and where the surface of each tissue is automatically calculated. Once the model is set up and validated, it can be applied to a new infrared image in only 1 minute. The infrared imaging technique is a solution to visualize on the same image the morphologic information and the molecular composition of tissues. It appears to be a helpful technique for studying objectively and quantitatively tumor response.

Our aim was to validate the use of IRMS to automate the recognition and the quantification of VX2 liver tumor viability after treatment with drug-eluting beads transarterial chemoembolization (DEB-TACE). We worked on a pool of untreated VX2 tumors and a pool of DEB-TACE-treated tumors. First, the infrared spectra characteristics of each tissue of interest were recorded and a prediction model of tissue types was developed. Second, the model was applied to a set of new test VX2 samples to assess the surface of viable and necrotic tumor. A validation procedure was included at each step of the data processing. Infrared results were correlated by histopathologic analysis as standard of reference.

Materials and Methods

Animal Model and Tissue Samples

This study was approved by the Animal Care Committee and was performed in accordance with our institutional guidelines. Adult New Zealand white rabbits weighing 6 to 8 lb underwent implantation of rabbit VX2 tumor in the liver. The tumors were induced by an injection of a VX2 cells suspension (0.25×10^6 cells/mL) directly in the liver. We included 27 rabbits with VX2 liver tumors: 16 rabbits were subjected to a DEB-TACE treatment and compared with a control group of 11 rabbits. Animals from DEB-TACE group were treated after 12 days of tumor development and were euthanized 3 days after the embolization procedure (15 days of tumor development). In the same model, one experiment on our laboratory found a significant increase of tumor necrosis 3 days after embolization compared with untreated tumors. Animals in the control group were euthanized after 14 days of tumor development. Then, tumor-bearing livers were resected and samples were formalin fixed and paraffin embedded. Two adjacent sections were cut from each sample using a microtome. The first section (10- μ m thick) was mounted on a calcium fluoride window suitable for IRMS. The second section (5- μ m thick) was put on a standard glass slide, dewaxed, and rehydrated by means of successive baths of xylene and alcohol and stained with hematoxylin-eosin-saffron (HES) to serve as a control for infrared imaging.

Fourier Transform IRMS

Infrared spectral images were collected with an infrared microscope (Spectrum Spotlight 300 Imaging System, Perkin-Elmer, Courtaboeuf, France) coupled to a Spectrum One Fourier transform infrared (FTIR) spectrometer using the image mode. The device is equipped with a nitrogen-cooled mercury cadmium telluride 16-pixel-line detector for imaging and a computer-controlled stage to collect large spectroscopic images from a sample. The microscope was isolated in a venting Plexiglas housing to enable purging with dry air and to eliminate atmospheric interferences. Before acquisition, a visible image of the sample was recorded and the area of interest was selected by comparison to the corresponding H&E-stained adjacent section.

In this study, 38 spectroscopic images were recorded from VX2 liver tumors and could vary in size from 8×8 mm (64 mm^2) to 10×10 mm (or 100 mm^2). Each pixel sampled a $25 \times 25 \text{ }\mu\text{m}$ ($625 \text{ }\mu\text{m}^2$) area at the sample plane, providing images that contained 120,000 to 160,000 individual infrared spectra (depending on the size of the image). Spectral data were acquired in transmission mode. All spectral measurements were recorded using a spectral resolution of 4 cm^{-1} and two scans per pixels, each spectrum containing 1601 values of absorbance, spanning the spectral range of 800 cm^{-1} to 4000 cm^{-1} . A background spectrum was collected (75 accumulations, 4-cm^{-1} resolution) outside the sample (on the calcium fluoride window

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