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Prostate Cancer

A Correlation of FTIR Spectra Derived from Prostate Cancer Biopsies with Gleason Grade and Tumour Stage

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Abstract

Objectives: We introduce biochemistry as a second dimension to Gleason grading, using Fourier transform infrared (FTIR) microspectroscopy. For the first time, we correlate FTIR spectra derived from prostate cancer (pCA) tissue with Gleason score and the clinical stage of the tumour at time of biopsy.
Methods: Serial sections from paraffin-embedded pCA tissue were collected. One was stained with hematoxylin and eosin and Gleason scored; FTIR spectra were collected from malignant locations using a second unstained section. FTIR spectra, representing different Gleason grades, were used to construct a diagnostic classifier for pCA using linear discriminant analysis (LDA). This model was blind tested using 383 IR spectra from 36 biopsies.
Results: Using a three-band Gleason criteria, we obtained sensitivity of $\geq$70% for the FTIR-LDA model to predict Gleason <7, $=7$, and >7, with specificities of $\geq$81%. Using a threshold of Gleason/FTIR-LDA score of $\geq$8, we obtained a sensitivity and specificity of 71% and 67%, respectively, for the correlation with metastatic tumours using the FTIR-LDA system and 85% and 63%, respectively, for the correlation of metastatic tumours using the Gleason system.
Conclusions: There is a correlation between tissue architecture using Gleason score with tissue biochemistry using FTIR-LDA. Both systems are similar in their performance in predicting metastatic behaviour in tumours from individual patients.

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1. Introduction

Prostate tumour assessment of biopsy material is predominantly carried out by the method of Gleason grading [1]. However, a limitation in this technique is the disparity between tissue architecture and biochemical progression, particularly after hormone manipulation [2]. Also, difficulties concerning the reproducibility of a specific Gleason score, due to intraobserver and interobserver variability, mitigate its acceptance as a single prognostic tool [3,4]. Despite the problems with Gleason grading, the majority of studies based on prostate cancer (pCA) prognosis use this system and some authorities have suggested that all new prognostic markers should be evaluated in conjunction with Gleason grade [5].

The concerns relating to Gleason grading have called for the development of diagnostic tools that operate on the basis of cellular biochemical rather than visual tissue architectural recognition. Vibrational microspectroscopy in the form of Fourier transform infrared (FTIR) and Raman spectroscopy, combined with imaging technology and multivariate statistical approaches to data analysis, have been much to the forefront in the development of practical diagnostic tools for the detection and cytopathologic grading of various neoplasms [6], such as pCA [7,8–11,12]. FTIR microspectroscopy measures the vibrational modes (mainly, stretching and bending) of functional groups of biomolecules as low-energy IR photons (0.05–0.5 eV) which are transmitted through the tissue, in vitro. The resulting IR spectrum is characteristic of the tissue's biochemical composition (lipid, protein, carbohydrate, and phosphorylated molecular domains).

Previously, our group has applied linear discriminant analysis (LDA) to FTIR spectra derived from paraffin-embedded pCA tissue of each Gleason grade (2–5), as a means of constructing an operator-independent diagnostic algorithm for pCA grading [10,11]. In the present study, we hypothesise that as the result of phenotypic heterogeneity within the population of malignant pCA cells, the FTIR-LDA grades generated from tumour lesions of pCA-biopsied tissue will differ from its associated Gleason grades. To test this hypothesis, we blind tested our FTIR-LDA diagnostic model with biopsies of known Gleason score and implemented analytical criteria that allow for the direct comparison of grades derived from each system. Secondly, we have correlated FTIR data, derived from tumour biopsies, with the clinical stage of these tumours (using the TNM system of prostate tumour classification) at the time of biopsy. Moreover, this correlation was used to investigate whether metastatic disease could be predicted through FTIR-LDA analyses.

2. Materials and methods

2.1. Primary tissue preparation and sampling for FTIR

Forty pCA tissue biopsy specimens were obtained as paraffin-embedded blocks (Genito-Urinary Cancer Research Group, Paterson Institute) from patients with pCA. With the exception of three biopsies that were radical prostatectomies and one transrectal ultrasound of the prostate, all samples were obtained with informed consent from men undergoing treatment for urinary outflow obstructive symptoms by transurethral resection in which there was prostate adenocarcinoma. Serial sections were collected at 10 μm thickness from each specimen, one of which was mounted onto a BaF_2 plate (Linkam Scientific) with the adjacent section mounted onto a glass slide and stained with hematoxylin and eosin (H&E). An experienced histopathologist (J.H.S.) assigned Gleason scores to areas of malignancy identified within the H&E sections. The complimentary sections, mounted onto BaF_2 plates, were washed on an orbital mixer with Citroclear for 6 min to remove the paraffin and then acetone at 4 °C for a further 6 min before being air-dried for 1 h under ambient conditions. The anatomic features identified from the H&E section were used as landmarks to position the IR beam on the malignant lesions of the unstained adjacent section.

2.2. FTIR microspectroscopy

FTIR spectra of Gleason-graded primary prostate tissues were collected in transmission mode using a Nicolet Magna system 550 spectrometer equipped with a liquid nitrogen cooled MCT/A detector and a KBr beam splitter. The spectrometer was attached to a microscope equipped with a video camera to view optical images (×150 magnification) of the sampling area and a programmable computerized x–y stage. An aperture size of 60 × 60 μm was used to collect malignant epithelial cell spectra. A simplified diagram of the FTIR microscope is shown in Fig. 1. FTIR spectra represent an average of 512 scans in the mid-IR wavenumber range 750–4000 cm^{-1} with a spectral resolution of 4 cm^{-1}. Background scans were obtained from a region of no sample and ratioed against the sample spectrum.

2.3. Data processing

IR spectra acquired from each Gleason-graded prostate tissue section were baseline corrected between the spectral region ~1720 cm^{-1} to ~980 cm^{-1} and normalised to the amide I (~1650 cm^{-1}) peak intensity, using the OMNIC v.5.1a software. Following this, the band region between 1481 cm^{-1} to 999 cm^{-1} (diagnostic spectral region) was used to construct or blind test the FTIR-LDA grading model using SPSS Release 11.0.0.

2.4. Statistical analysis

The Spearman rho, nonparametric (2-tailed) test was used to determine whether there was a significant correlation