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Raman spectroscopy of bladder tissue in the presence of 5-aminolevulinic acid

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ABSTRACT

Raman spectroscopy has the ability to provide differential diagnosis of different cancers with high sensitivity and specificity. A major limitation in its clinical application is the weak nature of Raman signal, which inhibits scanning large surface areas of tissues. In bladder cancer diagnosis, fluorescence-guided endoscopy with 5-aminolevulinic acid (5-ALA) has gained interest as a technique that can provide such spatial differentiation, thus improving early detection and more complete removal of superficial tumors. However, several studies have demonstrated the poor specificity of this modality. Combining fluorescence with Raman spectroscopy could improve its diagnostic capability. However, little is known about the effect of agents such as 5-ALA on Raman spectra of tissue. In this paper, we present measuring Raman spectroscopy from benign and malignant bladder tissues in the presence of 5-ALA and attempt to evaluate the potential to discriminate between different pathologies.

Raman spectra were recorded from 92 bladder biopsies without 5-ALA and 38 biopsies with 5-ALA using a Raman microspectrometer system at 830 nm excitation. Empirical and multivariate statistical techniques were used for data analysis. Algorithms were developed to determine the effect of 5-ALA on tissue and its influence on the prediction ability of a preliminary benign/malignant prediction model.

In samples with 5-ALA, an overall decrease in Raman intensity was observed when compared to the Raman spectra from samples without 5-ALA. Additionally, differences in relative intensities at 1270 and 1330 cm^{-1} were also noted. However, significant differences were observed in the Raman spectra of benign and malignant samples with 5-ALA indicating the potential of using Raman spectroscopy for discriminating bladder cancer in the presence of 5-ALA.

The Principal-Component fed Linear-Discriminant Analysis (PCA/LDA) algorithm derived from biopsies in the absence of 5-ALA used to predict biopsies in the presence of 5-ALA resulted in an overall sensitivity and specificity of 42.6% and 71.1%, respectively. This suggests the presence of 5-ALA in tissue affects the Raman spectra. A PCA/LDA algorithm based on fluorescence information (i.e. PpIX fluorescence positive or negative) and the Raman spectrum of 5-ALA biopsies, had a sensitivity and specificity of 100% and 80.8%, respectively.

This study demonstrates that applying 5-ALA affects the Raman spectra of bladder tissues. However, benign/malignant differentiation can be accomplished with a preliminary PCA/LDA algorithm, suggesting the potential of a combined diagnostic modality in vivo.

1. Introduction

The high prevalence of bladder cancer places a significant burden on the public health system. Over 1% of the population in the western world is diagnosed with it. With approximately 63,200 new cases per year in the US alone, it is the fourth most common cancer in men and the 9th in women [1]. Its incidence increases with age reaching a maximum between 65 and 75 years. Ninety percent of all bladder cancers is transitional cell carcinoma (TCC), which originates in the mucosa of the bladder wall [2]. It is generally accepted that there are separate biological pathways leading to bladder cancer which can be categorized into low risk patients with low-grade papillary tumors and a small chance of progression and high risk patients with carcinoma in situ (CIS) and high-grade neoplasia with high risk of progression to invasive disease [3,4].

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The standard of care in bladder cancer involves the removal of neoplastic tissue during transurethral resection of the tumor (TURT) and biopsy to assess the grade and stage of suspicious looking areas of the bladder wall during white light endoscopy (WLE). Additional treatment options for non-muscle invasive bladder cancer include intravesical therapy by instillation of immuno- or chemo therapy in the bladder or cystectomy.

Early stage bladder cancer, like CIS is typically restricted to the urothelium making diagnosis by conventional white light endoscopy difficult. Early detection and accurate TURT provides the best chance for patient survival. This has led to the development of techniques to increase the sensitivity for superficial bladder cancer diagnosis such as fluorescence imaging during cystoscopy.

1.1. Fluorescence imaging

Photodynamic diagnosis (PDD) or fluorescence-guided endoscopy (FGE) is optimized to reduce the number of overlooked papillary and flat neoplastic lesions in bladder cancer detection. Numerous studies have reported the increased sensitivity that FGE offers in detecting bladder cancer with the use of 5-aminolaevulinic acid (5-ALA) as a photosensitizing agent for FGE [5–7]. The increase in sensitivity is due to contrast enhancement, based on the difference in accumulation of protoporphyrin IX (PpIX) in tumor cells [8]. A number of processes, like enzymatic activity and the type of cell membrane transport of the PpIX precursor, contribute to the ratio of PpIX concentration in normal and tumor cells and fluorescence kinetics. The PpIX concentration in tumor cells yields a fluorescence contrast with normal tissue which is used for diagnosis [9].

In clinical practice, the benefit of 5-ALA induced PpIX fluorescence imaging of TCC in the bladder is demonstrated by the increase in the number of lesions found by FGE as compared to WLE and more importantly, by the decrease in recurrence rate of the disease [6,10,11]. Despite these advantages of FGE, the technique has not been embraced by the urologic community due to its high false positive rate. Most false positive fluorescent locations are due to benign conditions of the bladder wall, such as chronic inflammations and cystitis. However, some of these false positives may be due to genetic alterations that are similar to those found in tumor biopsies of the same patient, indicating early malignant changes not revealed by histopathology [12]. The false positive rate of 5-ALA guidance is known to increase when used shortly after intravesical immuno- or chemo therapy of the bladder [13]. This may be one of the reasons for the varying degree of success that is reported [14]. Therefore, there is a need to develop techniques that can enhance the specificity of bladder cancer diagnosis in vivo, thus reducing the false positive rate as well as increasing acceptance of optically guided tumor resection in urology.

1.2. Raman spectroscopy

Raman spectroscopy is an optical technique that utilizes inelastic scattering of light photons to interrogate the vibrational modes of materials. For many years, Raman spectroscopy has been used to explore the biochemistry of a variety of biological molecules due to its inherently molecular specific nature [15,16].

Several investigations have shown that Raman spectroscopy can successfully differentiate epithelial neoplasia from normal tissue and inflammation in many organs [17,18]. Molckovsky et al. demonstrated the diagnostic potential of Raman spectroscopy for differentiation of colonic polyps during endoscopy [19]. Shafer-Peltier et al. showed their biochemical model could be used to relate the Raman spectrum of a breast tissue sample to diagnostic parameters used by pathologists [20]. Raman spectroscopy has been applied to detect changes in the molecular composition of the bladder muscle [21] and by pathology-specific endothelial bladder tissues [22]. Robichaux et al. have shown a successful and minimally invasive application of Raman spectroscopy in the cervix [23].

The feasibility of Raman spectroscopy to categorize TCC of the bladder as high- or low-grade and non-invasive or invasive has been demonstrated in vitro, with a overall sensitivity and specificity of 92% and 97%, respectively, by means of a multivariate spectral classification [17]. Similar diagnostic capabilities have been reported by Crow et al. for in vitro measurements of bladder cancers using a fiber optic probe-based Raman system, which allows use in the operating room [24]. The application of Raman spectroscopy in vivo has been reported by numerous groups for organs such as cervix, breast, esophagus and skin, attesting to the potential of Raman spectroscopy as a viable tool to improve the specificity of in vivo tissue diagnosis [25–29].

Thus the combination of Raman spectroscopy with 5-ALA based fluorescence for the guided resection of bladder cancer is proposed where 5-ALA fluorescence imaging is used for large area screening and a Raman spectroscopy based algorithm is used for specific diagnosis at the region of interest. However, exogenous application of a precursor to induce fluorescence will affect the local tissue biochemistry as sampled by Raman spectroscopy. This may result in Raman spectral changes that complicate or fully prevent further diagnosis. Several studies may be required to determine whether this combined modality approach can result in a clinically feasible technique to improve the diagnosis of bladder cancer in vivo. This paper investigates the effect of 5-ALA on the Raman spectra of benign and malignant bladder tissues.

2. Materials and methods

Two sets of bladder samples were used to evaluate the influence of 5-ALA induced PpIX on tissue Raman spectra. Biopsy samples of the bladder wall were obtained from patients who underwent conventional white light cystoscopy (without 5-ALA) and fluorescence-guided cystoscopy (with 5-ALA). A total of 92 biopsies without 5-ALA was obtained from 73 patients undergoing TURT with conventional cystoscopy for known or suspected TCC of the bladder. These bladder samples were immediately snap-frozen with liquid nitrogen and stored at −80 °C until further Raman spectroscopic evaluation and histopathologic evaluation. A total of 38 samples with 5-ALA was obtained from 19 patients that underwent fluorescence-guided endoscopy. In these patients, 1.5 g of 5-ALA was dissolved in 50 ml of a 5.7% sodium monohydrogen phosphate buffer solution and instilled in the bladder 2 h prior to the surgical procedure. At the time of the surgical procedure, the bladder was inspected using conventional white light endoscopy followed by fluorescence inspection under blue light (375–405 nm). For this study, two additional biopsies were obtained with patient consent: one typically from an area with fluorescence and one from a non-fluorescent area. These biopsies were snap-frozen immediately in liquid nitrogen, placed in light tight cryogenic vials to prevent photo bleaching and stored at −80 °C before Raman spectral measurements.

At the time of Raman spectral measurements, each sample was thawed using saline and placed on a 1 × 3 in. calcium fluoride slide (a material known to have no Raman interference in the fingerprint region of the spectrum). The slide was placed on an upright microscope sample stage for Raman measurements. Care was taken to identify the urothelium and fatty tissue to ensure correct orientation with the urothelium facing up towards the laser beam. Raman spectra were obtained from 3 to 8 locations on each sample with an integration time of 10 s using a bench top micro-Raman system (Renishaw System 1000, Renishaw Inc., Gloucestershire, UK). The