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Changes in the microenvironment of invading melanoma and carcinoma cells identified by FTIR imaging

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ABSTRACT

Tumor microenvironment (TME) has become an important target for studying cancer progression in recent times. Disorientation of the collagen fiber network is a common phenomenon during cancer invasion process. In this study, using *in vitro* myoma organotypic model with invading melanoma and oral tongue carcinoma cell lines, we identified the influence of the cancer cells in the TME by Fourier-transform infrared (FTIR) imaging. We found major changes in the relative intensities of the collagen bands. Principal component analysis was performed to explore feasibility of classification between spectra extracted from different regions. A submolecular justification of the classification model was sought using a curve fitting analysis. Our preliminary results suggest that the features present in the amide and collagen triplet regions could serve as spectral markers for cancer-induced modifications in the TME. We suggest that FTIR method, combined with myoma invasion model, could be used to analyze various tumor cells interactions with TME during invasion processes.

1. Introduction

During metastasis, tumor cells defy the organ boundaries and go to distant sites in the body. The metastatic spread of cancer does not only depend on the tumor cells, but also on the interplay between cells and molecules in the environment surrounding the tumor defined as the tumor microenvironment (TME) [1,2]. The main function of TME is to provide nourishment to the cancer cells as they proliferate. In addition, TME is also an active participant during each step of carcinogenesis leading to metastases [3]. The main constituents of TME include blood vessels, fibroblasts, immune cells, signaling molecules, and the extracellular matrix (ECM). In the TME, the cancer-associated fibroblasts (CAFs) are a distinct group of myofibroblast-like fibroblasts which participate in the invasion progression of cancer cells [4,5].

The ECM, stores a variety of growth factors and bioactive molecules, which act as a barrier for cell invasion. In addition, ECM forms a scaffold for cell migration along its fibers and provide signaling cues to the cells *via* surface receptors [6,7]. Degradation of basement membranes and stromal ECM is crucial for invasion and metastasis of malignant cells [7,8]. In the invasion process, collagen network is commonly altered depending on the invasion mechanisms used by the malignant cells. For example, melanoma cells invade mainly using amoeboid pattern, whereas carcinoma cells degrade proteolytically ECM molecules, including reticular and collagen fibers [9,10].

Fourier-transform infrared spectroscopy (FTIR) is an absorption based optical spectroscopy technique, which is being projected as a

Abbreviations: ECM, Extra cellular matrix; FTIR, Fourier-transform infrared; OSCC, Oral squamous cell carcinoma; PC, Principal components; PCA, Principal component analysis; TME, Tumor microenvironment.

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powerful tool to study the structure, function and biochemical composition of tissues and cells. The IR absorption spectrum of biological cells and tissues depends on the concentrations of their various constituents, like proteins, DNA, RNA, lipids, sugars and metabolites. It also reflects a higher-level of information, like protein inventory, DNA and RNA structural organization, biomembrane composition and dynamics. Changes in the position and shape of bands associated with amide I ($\sim$1650 cm^{-1}), amide II ($\sim$1550 cm^{-1}), lipids and nucleic acids can be used to assess biochemical changes associated with disease onset. Over the past 3–4 decades, several technological advances have been made to facilitate FTIR imaging for biomedical applications [11–14]. Variety of biological specimens ranging from cell lines, blood cells, tissues, lymph system and stem cells have been probed with FTIR microspectroscopy [15–25]. Findings of all these studies have successfully indicated that FTIR imaging coupled with appropriate statistical data analysis approaches can be successfully applied to achieve high accuracy in classifying normal and pathological conditions [26].

Recently the major focus of the cancer research has inclined towards exploring the involvement of TME in cancer progression [27,28]. An accurate understanding of TME with respect to specific cell types may provide new targets for cancer treatment. Therefore, in the present study, we have evaluated the efficacy of FTIR imaging in identifying biological changes in the TME caused by melanoma and tongue carcinoma cells cultured on top of the TME mimicking human myoma organotypic 3D disc model. This hypoxic *in vitro* model provides invading cancer cells a natural human tumor ECM, including a variety of the stromal cells (non-vital during the assay) and all the extracellular matrix components present in TME [29,30].

2. Material and methods

2.1. Organotypic invasion assay

Melanoma and tongue carcinoma cell lines were cultured on the top of 3D myoma discs as described previously (Fig. 1) [29]. Briefly, uterine leiomyoma tissue was retrieved from routine surgical operations after obtaining informed consent form signed by the donating patients. The data inquiry was approved by the Ethics Committee of the Northern Ostrobothnia Hospital District. Four (4) mm thick and 8 mm-diameter myoma disks were prepared and stored at −70 °C in media with 10% DMSO (Sigma–Aldrich). As there are differences between myoma tissues, all these experiments were done using disks from the same myoma. To prepare myoma-based organotypic cultures, myoma disks were equilibrated in culture medium at room temperature for 1 h, after which cancer cells were added on top of each disk.

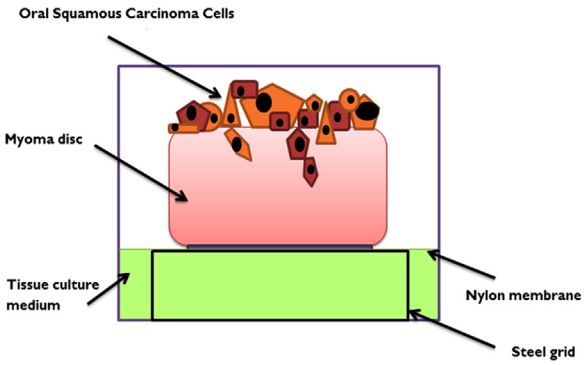

Fig. 1. Schematic drawing of the 3D organotypic human myoma invasion assay where the carcinoma cells are cultured on top of the myoma tissue for 10 days (modified from Professor Tuula Salo's archives).

Human tongue SCC cells HSC-3 (JCRB 0623; Osaka National Institute of Health Sciences, Osaka, Japan) were cultured in 1:1 Dulbecco's modified Eagle's medium (DMEM)/F-12 (Invitrogen, Carlsbad, CA) supplemented with 100 U/mL penicillin, 100 g/mL streptomycin, 50 g/mL ascorbic acid, 250 ng/mL fungizone, 5 μg/mL insulin (bovine pancreas), 0.4 ng/mL hydrocortisone (all reagents from Sigma–Aldrich, St. Louis, MO, USA), and 10% heat-in-activated fetal bovine serum (Perbio Science, Erembodegem, Belgium). Melanoma cell lines Bowes (ATCC CRL-9607) and G361 (ATCC CRL-1424) were cultured in DMEM supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin, 50 μg/mL ascorbic acid, 250 ng/mL fungizone, 1 mmol/L sodium pyruvate (all from Sigma–Aldrich), and 10% heat-in-activated fetal bovine serum.

Two melanoma cell lines (Bowes and G361) and the HSC-3 tongue carcinoma cell line were cultured for 10 days in the organotypic model. The disks were fixed in 4% neutral-buffered formalin overnight. The specimens were dehydrated, bisected, and embedded in paraffin. Sections (5 μm) were deparaffinized and stained with Mayer's H&E. To identify the invasive cells and to measure the invasion depth according to the method described by Nurmenniemi et al. [29], myoma specimens were stained as follows: the melanoma cells with S100, and the HSC-3 cells with pan-cytokeratin AE1/AE3 as described previously [29].

2.2. FTIR imaging

2.2.1. Sample preparation

The formalin fixed and paraffin embedded organotypic model TME blocks with invading two melanoma and one tongue carcinoma cell were analyzed. The spectroscopic imaging experiments to identify cells induced changes to the organotypic TME model were carried out in transmission mode on unstained and deparaffinized 4 μm thick tissue slices deposited on 40 × 26 mm^2 BaF_2 slides. Sections were rehydrated before the FTIR imaging.

2.2.2. Data acquisition and processing

Hyperion 3000 FTIR imaging system (Bruker Optics, Ettlingen, Germany) equipped with a 64 × 64 MCT (Mercury–Cadmium–Telluride) 2560 × 2560 μm^2 FPA (focal plane array) detector with a 15× magnification was used to acquire the FTIR data. The spectral resolution was set to 8 cm^{-1} and the data were acquired in transmission mode from sample regions of 170 × 170 μm^2 for each unit image. Each individual element of the array detector sampled an area of 2.7 × 2.7 μm^2. One IR image measurement resulted in 4096 spectra, each one being the average of 256 scans recorded in 4–5 min. The experiments were performed at room temperature using the spectrometer software OPUS. Each single beam spectrum was ratioed against a background spectrum obtained in the absence of the sample and converted to absorbance by the OPUS software. Larger mapping area (typically 3 × 3 or 3 × 4 unit images) was usually achieved. All the data processing and analysis were preformed by home grown program Kinetics and running under Matlab (Mathworks Inc., Natick, Ma, USA). Before statistical analysis spectra were preprocessed for baseline and water vapor corrections as described previously [14,31,32,34]. The water vapor contribution was subtracted as described previously with 1956–1935 cm^{-1} as reference peak [14,32]. Spectra were normalized for equal area between 1725 and 1481 cm^{-1}. An 11-point baseline passing by 3620, 2995, 2800, 2395, 2247, 1765, 1724, 1480, 1355 and 1200 cm^{-1} was subtracted [14,31,32]. Also Mie scattering corrections and normalization were performed to avoid the any