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ORIGINAL ARTICLE

Quantitative Analysis on Ex Vivo Nonlinear Microscopy Images of Basal Cell Carcinoma Samples in Comparison to Healthy Skin

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Abstract
Basal cell carcinoma (BCC) is the most frequent malignant neoplasm in the Caucasian population. There are several therapeutic options for BCC, but surgical excision is considered gold standard treatment. As BCCs often have poorly defined borders, the clinical assessment of the tumor margins can be challenging. Therefore, there is an increasing demand for efficient *in vivo* imaging techniques for the evaluation of tumor borders prior to and during surgeries. In the near future, nonlinear microscopy techniques might meet this demand. We measured the two-photon excitation fluorescence (TPEF) signal of nicotinamide adenine dinucleotide hydride (NADH) and elastin and second harmonic generation (SHG) signal of collagen on 10 *ex vivo* healthy control and BCC skin samples and compared the images by different quantitative image analysis methods. These included integrated optical density (IOD) measurements on TPEF and SHG images and application of fast Fourier transform (FFT), CT-FIRE and CurveAlign algorithms on SHG images to evaluate the collagen structure. In the BCC samples, we found significantly lower IOD of both the TPEF and SHG signals and higher collagen orientation index utilizing FFT. CT-FIRE algorithm revealed increased collagen fiber length and decreased fiber angle while CurveAlign detected higher fiber alignment of collagen fibers in BCC. These results are in line with previous findings which describe pronounced changes in the collagen structure of BCC. In the future, these novel image analysis methods could be integrated in handheld nonlinear microscope systems, for sensitive and specific identification of BCC.

Keywords Basal cell carcinoma · Nonlinear microscopy · Second-harmonic generation · Collagen structure · Quantitative analysis

Introduction

Basal cell carcinoma (BCC) is the most frequent malignant neoplasm in the Caucasian population [1]. The incidence of BCC varies greatly worldwide, with the highest rates in Australia (>1000/100000 person-years) and the lowest rates in parts of Africa (<1/100000 person-years) [2]. The most important risk factors for BCC include fair skin phenotype and excessive occasional sun exposure [3]. Although the metastatic rate of BCC is very low, it can lead to significant tissue destruction by local invasion to result in major cosmetic damage and inoperable propagation, thus early diagnosis of BCC is crucial [4].

There are several therapeutic options for BCC, including cryo-, photodynamic- and radiotherapy [5], but surgical excision is considered gold standard. Among surgical techniques, Mohs micrographic surgery (MMS) provides the lowest rate of tumor recurrence with the smallest resected area by utilizing repeated microscopic examinations of frozen sections of the tumor margins during surgery. However, MMS is a time-consuming technique that also requires special expertise. As BCCs often have poorly defined borders, without utilizing MMS, the clinical assessment of the tumor margins can be challenging [6]. Therefore, there is an increasing demand for efficient *in vivo* imaging techniques for the evaluation of the tumor borders of BCC prior to and during surgeries. In the near future, nonlinear microscopy techniques might meet this demand [7].

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