Check for updates

BRIEF REPORT

Potential use of absorbance scanning in differentiating tumor microenvironment compositions from endoscopic biopsy of colorectal cancer patients. [version 1; peer review: 1 not approved]

Murdani Abdullah ^{1,2}, Amanda Pitarini Utari¹, Saskia Aziza Nursyirwan ¹, Dimas R. Noor²

¹Department of Internal Medicine, Faculty of Medicine, Faculty of Medicine Universitas Indonesia, GH Building, Jalan Diponegoro No. 71, Kenari, Senen, Jakarta, 10430, Indonesia

²Human Cancer Research Center, Indonesia Medical Education and Research Institute, Faculty of Medicine Universitas Indonesia,, Jalan Salemba Raya No.6, RW.5, Kenari, Senen, Jakarta, 10430, Indonesia

V1 First published: 21 Dec 2021, 10:1300
<https://doi.org/10.12688/f1000research.74480.1>
Latest published: 21 Dec 2021, 10:1300
<https://doi.org/10.12688/f1000research.74480.1>

Abstract

Background: The microtumor environment is an area where tumor cells are surrounded by several cells such as immune cells, stromal cells and endothelial cells as well as blood and lymphatic vessels. It is known that various cytokines and growth factors are released by various cells surrounding the tumor and affect the growth of tumor cells. Differences in chemical composition in the microtumor environment can be detected using spectrometry. The aim of this study was to examine the potential of scanning absorbance spectrometry in differentiating cells based on the chemical composition of endoscopic biopsies of colorectal cancer patients.

Methods: An endoscopic biopsy of the patient from Cipto Mangunkusumo Hospital, Jakarta was collected and homogenized in phosphate buffer saline using TissueLyser. Scanning absorbance was performed using a UV-VIS spectrophotometer. The results of scanning absorbance were then processed using principal component analysis (PCA) in Orange Data-Mining version 3.28.0 software and the results were represented in a scatter plot diagram.

Results: Based on the results of the analysis using PCA, three patients were identified in different quadrant regions, crc006 and crc011 obtained from patients with the differentiation status located close together while crc009 and undifferentiated were located far apart.

Conclusion: This indicates the potential of scanning absorbance in differentiating the chemical composition of the tumor microenvironment from patient biopsies.

Open Peer Review

Approval Status

1

version 1

21 Dec 2021

[view](#)

1. **Cornelis F. M. Sier** , Leiden University
Medical Center, Leiden, The Netherlands

Any reports and responses or comments on the article can be found at the end of the article.

Keywords

absorbance scanning, tumor microenvironment, compositions, endoscopic biopsy, colorectal cancer

This article is included in the **Oncology** gateway.

Corresponding author: Murdani Abdullah (kolitgastro@gmail.com)

Author roles: **Abdullah M:** Conceptualization, Funding Acquisition, Supervision, Writing – Original Draft Preparation, Writing – Review & Editing; **Pitarini Utari A:** Funding Acquisition, Resources, Writing – Original Draft Preparation, Writing – Review & Editing; **Aziza Nursyirwan S:** Funding Acquisition, Resources, Writing – Original Draft Preparation, Writing – Review & Editing; **Noor DR:** Methodology, Writing – Original Draft Preparation

Competing interests: No competing interests were disclosed.

Grant information: We thank HIBAH PUTI Q2 Universitas Indonesia (NKB-1540/UN2.RST/HKP.05.00/2020) for funding our study. *The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.*

Copyright: © 2021 Abdullah M *et al.* This is an open access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Abdullah M, Pitarini Utari A, Aziza Nursyirwan S and Noor DR. **Potential use of absorbance scanning in differentiating tumor microenvironment compositions from endoscopic biopsy of colorectal cancer patients. [version 1; peer review: 1 not approved]** F1000Research 2021, 10:1300 <https://doi.org/10.12688/f1000research.74480.1>

First published: 21 Dec 2021, 10:1300 <https://doi.org/10.12688/f1000research.74480.1>

Introduction

The tumor microenvironment is an area surrounding a tumor consisting of various cells including stromal cells (Wang *et al.*, 2017), immune cells (Winkler *et al.*, 2020), endothelial cells, blood, and lymph vessels (Wei *et al.*, 2020). The intra-tumoral composition of the tumor microenvironment can influence the tumor progression (Whiteside, 2008) and the clinical outcome of patients. To be able to determine the intra-tumoral composition, one common method is histopathological evaluation of the patient's biopsy (Schillaci *et al.*, 2019). However, the biochemical composition of the biopsy such as metabolites and cytokines requires further tests such as immunohistochemistry of cell markers. (Oudijk *et al.*, 2015) On the other hand, tumor progression may be influenced by chemical compositions within the tumor microenvironment such as cytokines and growth factors (Landskron *et al.*, 2014; Yuan *et al.*, 2016).

One of the simple methods that can be used to distinguish chemical compositions is spectrometry and potential differences of chemical compositions in malignancy such as changes in amino acid concentrations (Balan *et al.*, 2019). Previously, Nogueira *et al.*, (2021) showed that a tumor can be detected using diffuse reflectance spectroscopy in colorectal cancer diagnosis. Another study by Yang *et al.*, (2016) showed the potentiality of visible-absorption spectroscopy as a biomarker to predict treatment and prognosis of esophageal cancer. In this study, we report absorbance scanning to distinguish three biopsies from three different patients. The absorbance scanning was carried out at a wavelength of 400–1000 nm using a spectrophotometer followed by Principal Component Analysis Orange Data Mining.

Methods

Patients and sample collection

All subjects gave their written informed consent for inclusion before participating in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethical Committee of the Faculty of Medicine, Universitas Indonesia (Protocol ID: 20-04-0643, version 02, June 29th, 2020). The tissue samples taken from an endoscopic biopsy from patients from Cipto Mangunkusumo Hospital, Jakarta were confirmed as cancer by an anatomic pathologist in the Department of Pathological Anatomy, Faculty of Medicine, Universitas Indonesia. They were then put into transport media in phosphate buffer saline (PBS) and transferred to the laboratory of the Indonesia Medical Education and Research Institute, Faculty of Medicine, Universitas Indonesia in less than two hours.

Tissue extractions

The samples were minced into smaller tissue fragments, transferred to 1.5 ml centrifuge tubes, and homogenized by TissueLyser II (Qiagen, Germany) for 10 minutes. The suspensions were diluted in PBS to 1 mL.

Absorption spectrum profiles of the biopsy

The samples underwent wavelength scanning at $\lambda = 400\text{--}1000$ nm using the Agilent Cary 60 UV-Vis spectrophotometer (Agilent, Unites States). The absorption spectrum of the lysate cell was examined and feature selection was carried out using principal component analysis (PCA) to simplify the wavelength dimensions into principal component (PC) features that were not correlated (Abdullah *et al.*, 2021). The resulting PC features were then used to classify the cancer patient samples into a scatterplot. The absorption/absorption spectrum pre-processing and PCA analysis were performed using Orange Data-Mining version 3.28.0 (Orange Data Mining, RRID: SCR_019811) (Demšar *et al.*, 2013).

Results

In this study, we recruited three different patients namely CRC006, CRC009, and CRC011. In the samples collected from patients, CRC006 and CRC011 similar clinical characteristics such as histopathology results were observed. Well-differentiated rectal adenocarcinoma with metastatic sites were found in both patients. The sample collected from patient CRC009 had different clinical characteristics compared to CRC006 and CRC011, and CRC009 was found to have undifferentiated adenocarcinoma without metastasis. Sample data are shown in Table 1. (Abdullah *et al.*, 2021)

Figure 1 shows that each cell produces an absorption spectrum fingerprint in the wavelength range of 400–1000 nm. Just by observing the form of the absorption/absorption spectrum, it can be seen at first glance that fingerprints were only contributed to by the concentration of the molecule. To investigate the characteristic features of the spectrum, a principal component analysis (PCA) was carried out, which resulted in PC1 as the main feature (Abdullah *et al.*, 2021). The results of the scatterplot with PC1 and PC2 showed that the sample CRC011 was well grouped in quadrant 1, CRC006 in quadrant 4, while CRC009 was spread between quadrants 2 and 3. From Figure 1 it can also be seen that each type of sample group appears to experience separation/classification when compared to other groups.

Discussion

The light absorption spectrum is known as a “molecular fingerprint” which allows the analysis of molecular types and their amounts/concentrations. In Table 1, we show three representative colorectal cancer biopsies collected

Table 1. Patient clinical and histopathology profiles.

Sample ID	Histopathology results	Metastasis
CRC006	Well differentiated rectal adenocarcinoma	Yes
CRC009	Undifferentiated adenocarcinoma	No
CRC011	Well differentiated adenocarcinoma	Yes

Figure 1. Absorbance spectrum of the lysate cells scanned using nanospectrophotometers from 400–1000 nm. CRC: sample number. **Figure 2.** Scatterplot of cell suspensions from samples taken from patients CRC006, CRC009, and CRC011 (n = 3) based on PC1 and PC2 values.

endoscopically from patients: CRC006 was a patient with well-differentiated rectal adenocarcinoma with metastasis, CRC009 was from a patient with undifferentiated adenocarcinoma, CRC011 was from a patient with well-differentiated adenocarcinoma with metastasis. Interestingly, based on the PCA analysis of the absorption spectrum of the lysate sample (shown in [Figure 2](#)), the CRC006 quadrant was closer to CRC011, which has similar clinical features of well-differentiated adenocarcinoma and metastasis. On the other hand, CRC009 with undifferentiated and located far from CRC006 and CRC011 respectively, which indicated their closer chemical composition. Such existence of grouping indicates that there were differences in cellular composition between the three samples; although further studies with a larger number of samples are needed to further confirm the clustering that occurs.

In biological tissue samples, several molecules contribute to the absorption spectrum in the ultraviolet (UV) to near-infrared (NIR) regions. Protein and DNA absorb in the UV region, whereas tryptophan, NAD⁺ (nicotinamide adenine dinucleotide), collagen, elastin, NADH, and FAD (flavin adenine dinucleotide) are important endogenous chromophores in biological tissue ([Yang et al., 2016](#)). FAD, tryptophan, and NADPH (nicotinamide adenine dinucleotide phosphate) have been used as a measuring tool to see the mitochondrial metabolic response before and after therapy such as in prostate cancer ([Alam et al., 2017](#)). In cell lysate samples such as in this study, further molecular analysis is needed to determine the differences in the composition of compounds in the absorption/absorption spectrum between lysates from cancer patients.

Conclusions

The combinations of principal component analysis (PCA) and absorbance scanning were able to group patients with similar histopathology and metastasis based on their chemical compositions. Although further investigation is needed, molecular fingerprinting using absorbance scanning to predict intratumoral similarity and difference is exciting.

Data availability

Underlying data

Open Science Framework: Underlying data for 'Potential use of absorbance scanning in differentiating tumor micro-environment compositions from endoscopic biopsy of colorectal cancer patients available at <https://doi.org/10.17605/OSF.IO/QV7H5> ([Abdullah et al., 2021](#)).

This project contains the following underlying data:

- Absorbance – Orange data.docx
- UV-Vis Spectrum data.xlsx

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](#) (CC-BY 4.0).

Consent

Written informed consent for publication of the patients' details was obtained from the patients.

Grant information

We thank HIBAH PUTI Q2 Universitas Indonesia (NKB4127/UN2.RST/HKP.05.00/2020) for funding our study.

Acknowledgements

We thank Dr Aryo Tedjo from the Department of Medical Chemistry and Drug Development Cluster for supporting the absorbance scanning and Orange mining data analysis.

References

Abdullah M, Utari AP, Nursyirwan S, et al.: **Potential Use of Absorbance Scanning in Differentiating Tumor Microenvironment Compositions from Endoscopic Biopsy of Colorectal Cancer Patients.** 2021.
[Publisher Full Text](#)

Alam SR, Wallrabe H, Svindrych Z, et al.: **Investigation of Mitochondrial Metabolic Response to Doxorubicin in Prostate Cancer Cells:**

An NADH, FAD and Tryptophan FLIM Assay. *Sci. Rep.* 2017; **7**: 10451.
[PubMed Abstract](#) | [Publisher Full Text](#)

Balan V, Mihai C-T, Cojocaru F-D, et al.: **Vibrational Spectroscopy Fingerprinting in Medicine: from Molecular to Clinical Practice.** *Materials.* 2019; **12**.
[PubMed Abstract](#) | [Publisher Full Text](#)

Demšar J, Curk T, Erjavec A, *et al.*: **Orange: Data Mining Toolbox in Python.** *J. Mach. Learn. Res.* 2013; **14**: 2349–2353.

Landskron G, De la Fuente M, Thuwajit P, *et al.*: **Chronic inflammation and cytokines in the tumor microenvironment.** *J. Immunol. Res.* 2014; **2014**: 149185–149185.

[PubMed Abstract](#) | [Publisher Full Text](#)

Nogueira MS, Maryam S, Amissah M, *et al.*: **Evaluation of wavelength ranges and tissue depth probed by diffuse reflectance spectroscopy for colorectal cancer detection.** *Sci. Rep.* 2021; **11**: 798.

[PubMed Abstract](#) | [Publisher Full Text](#)

Oudijk L, Neuhofer CM, Lichtenauer UD, *et al.*: **Immunohistochemical expression of stem cell markers in pheochromocytomas/paragangliomas is associated with SDHx mutations.** *Eur. J. Endocrinol.* 2015; **173**: 43–52.

[PubMed Abstract](#) | [Publisher Full Text](#)

Schillaci O, Scimeca M, Toschi N, *et al.*: **Combining Diagnostic Imaging and Pathology for Improving Diagnosis and Prognosis of Cancer.** *Contrast Media Mol. Imaging.* 2019; **2019**: 1–10.

[PubMed Abstract](#) | [Publisher Full Text](#)

Wang M, Zhao J, Zhang L, *et al.*: **Role of tumor microenvironment in tumorigenesis.** *J. Cancer.* 2017; **8**: 761–773.

[PubMed Abstract](#) | [Publisher Full Text](#)

Wei R, Liu S, Zhang S, *et al.*: **Cellular and Extracellular Components in Tumor Microenvironment and Their Application in Early Diagnosis of Cancers.** *Anal. Cell. Pathol.* 2020; **2020**: 6283796–6283713.

[PubMed Abstract](#) | [Publisher Full Text](#)

Whiteside TL: **The tumor microenvironment and its role in promoting tumor growth.** *Oncogene.* 2008; **27**: 5904–5912.

[PubMed Abstract](#) | [Publisher Full Text](#)

Winkler J, Abisoye-Ogunniyan A, Metcalf KJ, *et al.*: **Concepts of extracellular matrix remodelling in tumour progression and metastasis.** *Nat. Commun.* 2020; **11**: 5120.

[PubMed Abstract](#) | [Publisher Full Text](#)

Yang P-W, Hsu I-J, Chang C-W, *et al.*: **Visible-absorption spectroscopy as a biomarker to predict treatment response and prognosis of surgically resected esophageal cancer.** *Sci. Rep.* 2016; **6**: 33414–33414.

[PubMed Abstract](#) | [Publisher Full Text](#)

Yuan Y, Jiang Y-C, Sun C-K, *et al.*: **Role of the tumor microenvironment in tumor progression and the clinical applications (Review).** *Oncol. Rep.* 2016; **35**: 2499–2515.

[Publisher Full Text](#)

Open Peer Review

Current Peer Review Status:

Version 1

Reviewer Report 28 March 2022

<https://doi.org/10.5256/f1000research.78241.r126731>

© 2022 Sier C. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Cornelis F. M. Sier

Department of Surgery, Leiden University Medical Center, Leiden, The Netherlands

The brief report by Murdani Abdullah and co-authors reports rather preliminary looking results of PBS- homogenates from biopsy material from 3 colorectal cancer patients which were scanned in a UV-Vis spectrophotometer from 400-1000 nm. Although the introduction focuses slightly on the tumor microenvironment there is no actual research question formulated. The cohort of samples (n=3) is definitely not enough to draw conclusions about the applicability of the method. In conclusion, this version of the manuscript does not contribute to the scientific literature.

Minor remarks:

- The word potential in the title seems rather opportunistic
- Next to a larger and more defined cohort, tissue biopsies are often rather superficial and might not represent the whole tumor.
- The authors could/should consider including normal tissue adjacent to the tumor to establish the difference with the tumor within single patients.
- Homogenates and lysates are not synonyms, the latter usually contains a detergent.
- PCA analysis should be better explained.
- The table needs to be extended, legends of figures need revision.

Is the work clearly and accurately presented and does it cite the current literature?

No

Is the study design appropriate and is the work technically sound?

No

Are sufficient details of methods and analysis provided to allow replication by others?

No

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: biomarkers in oncology, including colorectal cancer

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com

F1000Research