ORAL MICROBIOME AS NEW CLUE TO UNDERSTANDING COLORECTAL CANCER?

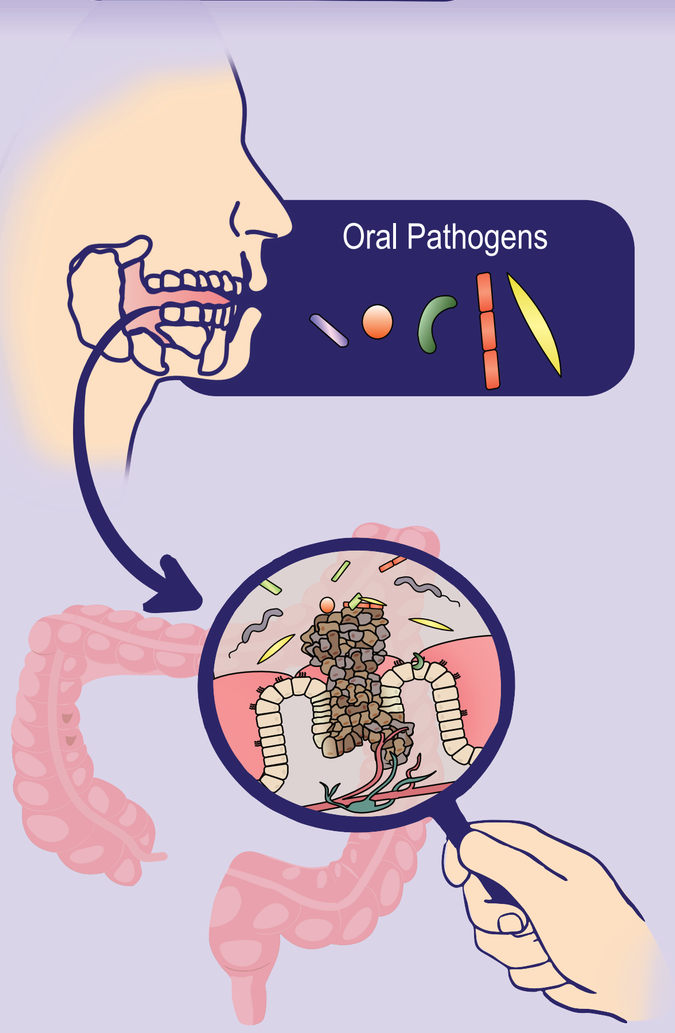
Martina Hrivňáková [1], Lenka Micenková [1], Barbora Zwinsová [1], Eva Budinská [1]

1. Research Centre for Toxic Compoundsin the Environment, Masaryk University, Kamenice 5, 625 00 Brno, CzechRepublic

INTRODUCTION

Colorectal cancer (CRC) is one of the most common cancers worldwide. Although the numbers of colorectal cancer are currently slightly decreasing in developed countries, mainly thanks to the preventive and screening programs, it is still a disease with complicated treatment and high mortality grade. Microbiome associated studies indicated that a state of pathological microbial imbalance or dysbiosis is prevalent in the gut of patients with colorectal cancer (CRC). Although there is a significant increase in knowledge in these fields of study, no single species has been found as a perfect marker universally present among patients with CRC. Even more, there is significant variation in microbial composition between CRC patients. The complexity of the microbiome turns the need for microbial marker-based diagnosis techniques into a real challenge.

BACKGROUND



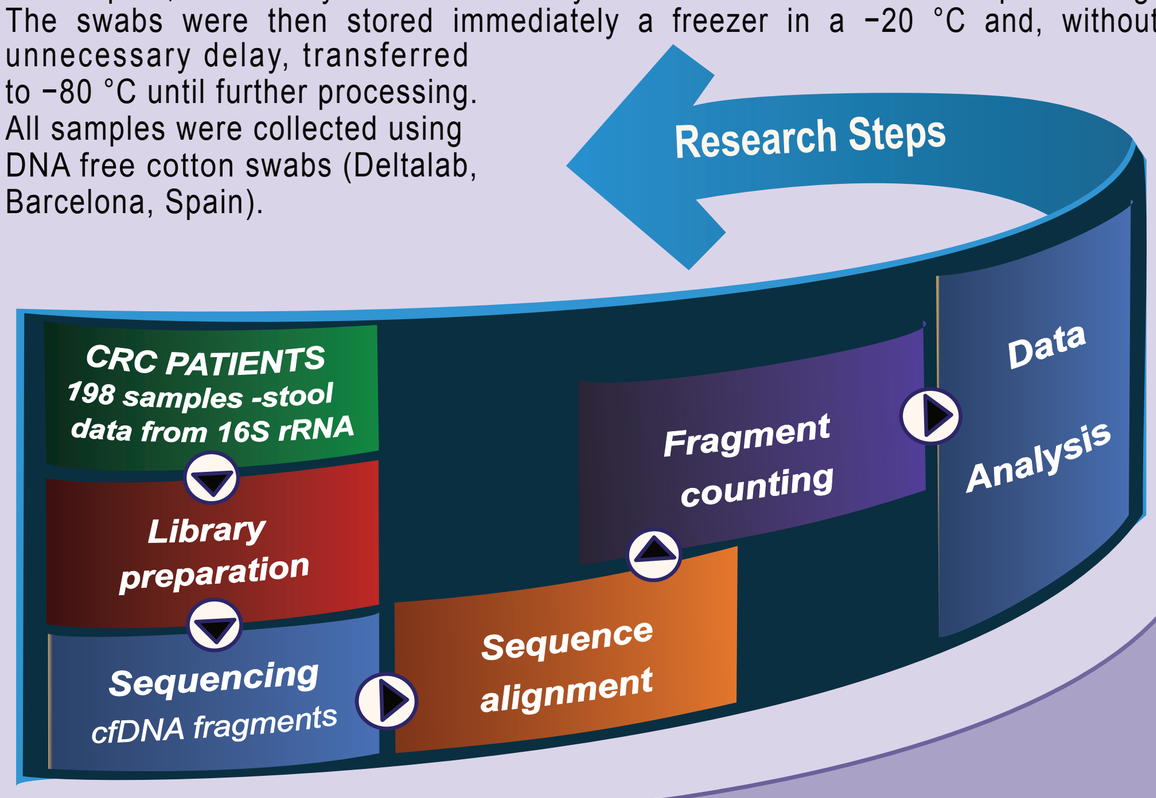
Microbiota alterations are linked with colorectal cancer and a notably higher abundance of putative oral bacteria in colonic tumours.

Multiple factors make the CRC tumour niche a favourable environment for oral bacteria, in particular, for oral pathogens (1, 2, 3). Inflammation in the oral tissue niche selects for those species that are most adapted to the new environment, producing specific molecules such as microbial proteolytic enzymes (4) that break down the host's extracellular matrix and soluble factors to get nutrients and invade the tissue.

Previously analyzed data obtained from 16S rRNA gene sequencing of paired tumour mucosa, adjacent visually normal mucosa and stool swabs showed that tumour mucosa is dominated by pathogenic bacteria of oral origin (5). However 16S rRNA gene sequencing is useful for the identification of bacterial genera, but it fails at the identification of strain level, in contrast to metagenomic and metatranscriptomic approaches.

METHODS AND SAMPLES

All specimens were collected at Masaryk Memorial Cancer Institute (Brno, Czech Republic) from 2015 to 2019. The stool samples were collected from untreated patients before the scheduled surgery. Patients performed the collection at home, on the morning of their hospitalization for the surgery and brought the samples to the hospital, where they were immediately frozen at −80 °C until further processing. The swabs were then stored immediately a freezer in a −20 °C and, without unnecessary delay, transferred to −80 °C until further processing. All samples were collected using DNA free cotton swabs (Deltalab, Barcelona, Spain).

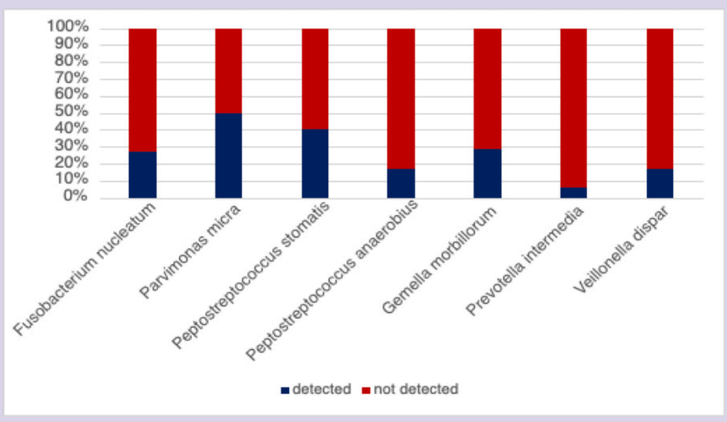


RESULTS AND DISCUSSION

In this study, we looked at specific oral bacteria as a potential marker for cancer stratification. Fusobacterium nucleatum is one of the most described markers for CRC. However, detection among colorectal cancer patients is highly inconsistent (Table 1.) but still is considered the best potential marker for CRC detection.

Publication	Sample	Population	% of detection *F. nucleatum*	References
Bundgaard-Nielsen et al.	tissue	Denmark	29,3 %	(6.)
Eklöf et al.	stool/tissue	Sweden	69,2 %	(7.)
Ito et al.	tissue	Japan	56 %	(8.)
Li et al.	tissue	China	22 %	(9.)
Mima et al.,	tissue	USA	13 %	(10.)
Yamaoka et al.	tissue	Japan	75 %	(11.)
Our data	stool	Czechia	26,77 %	

Table 1. Studies examining the presence of *F. nucleatum* in CRC samples based on population and sample type



Moreover, other oral bacteria showed very similar or worse coverage detection rate among patients with CRC (Fig.1). Better results may not depend on finding one reliable and powerful prognostic bacteria, but rather on more specific bacteria as a panel (Fig.2).

Fig 1. The percentage of detection oral bacteria (*Fusobacterium nucleatum, Parvimonas micra, Peptostreptococcus stomatis, peptostreptococcus anaerobius, Gemella morbillorum, Prevotella intermedia and Veillonella dispar)*

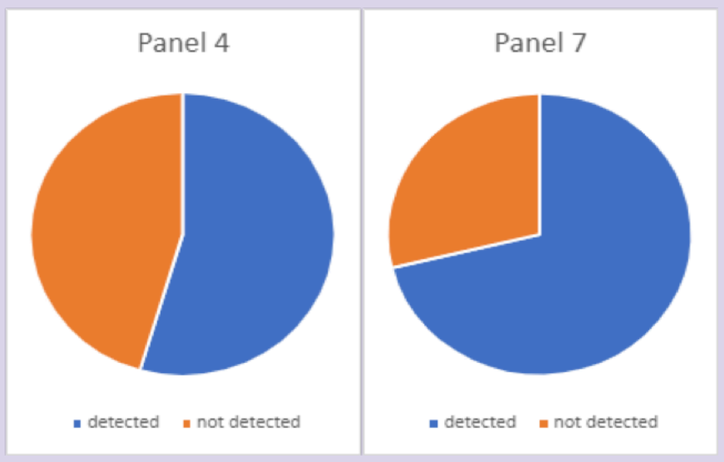


Fig 2. Differences in percentages of detected bacteria among CRC patients based on different Panels. Panel 4 is based on the 4 most known bacteria, and Panel 7 is based on all 7 most known bacteria across the literature.

CONCLUSION

- The heterogeneity of CRC may relate to the host-microbiome that either predisposes or provide resistance to the disease and profiling the oral microbiome may offer an alternative screen for detecting CRC.
- Considering more specific bacteria would be beneficial for more patients.
- Next step will be identification specific taxa on tumour samples of CRC patients.

ACKNOWLEDGEMENT:

Authors thanks to Research Infrastructure RECETOX RI (No LM2018121) financed by the Ministry of Education, Youth and Sports, and Operational Programme Research, Development and Innovation—project CETOCOEN EXCELLENCE (No CZ.02.1.01/0.0/0.0/17_043/0009632) for supportive background. This work received funding from the Ministry of Health of the Czech Republic (project AZV 16-31966A) and the European Union's Horizon 2020 research and innovation programme under grant agreement No 825410. This publication reflects only the author's view and the European Commission is not responsible for any use that may be made of the information it contains. Computational resources were supplied by the project "e-Infrastruktura CZ" (e-INFRA LM2018140) provided within the program Projects of Large Research, Development, and Innovations Infrastructures. The work was supported by the project BBMRI-CZ no. LM2018125.

REFERENCES

1. Long X., Wong C.C., Tong L., Chu E.S.H., Szeto C.H., Go M.Y.Y., Coker O.O., Chan A.W.H., Chan F.K., Sung J.J.Y., et al. Peptostreptococcus anaerobius promotes colorectal carcinogenesis and modulates tumour immunity. Nat. Microbiol. 2019;4:2319–2330. doi: 10.1038/s41564-019-0541-3. 2. Abed J., Emgård J.E., Zamir G., Faroja M., Almogy G., Grenov A., Sol A., Naor R., Pikarsky E., Atlan K.A., et al. Fap2 Mediates Fusobacterium nucleatum Colorectal Adenocarcinoma Enrichment by Binding to Tumor-Expressed Gal-GalNAc. Cell Host Microbe. 2016;20:215–225. doi: 10.1016/j.chom.2016.07.006. 3. Zou X., Feng B., Dong T., Yan G., Tan B., Shen H., Huang A., Zhang X., Zhang M., Yang P., et al. Up-regulation of type I collagen during tumorigenesis of colorectal cancer revealed by quantitative proteomic analysis. J. Proteom. 2013;94:473–485. doi: 10.1016/j.jprot.2013.10.020. 4. Takahashi N. Microbial ecosystem in the oral cavity: Metabolic diversity in an ecological niche and its relationship with oral diseases. Int. Congr. Ser. 2005;1284:103–112. doi: 10.1016/j.ics.2005.06.071. 5. Zwinsová, Barbora et al. "Colorectal Tumour Mucosa Microbiome Is Enriched in Oral Pathogens and Defines Three Subtypes That Correlate with Markers of Tumour Progression." Cancers vol. 13,19 4799. 25 Sep. 2021, doi:10.3390/cancers13194799. 6. Bundgaard-Nielsen, C., Baandrup, U.T., Nielsen, L.P. et al. The presence of bacteria varies between colorectal adenocarcinomas, precursor lesions and non-malignant tissue. BMC Cancer 19, 399 (2019). https://doi.org/10.1186/s12885-019-5571-y. 7. Eklöf V, Löfgren-Burström A, Zingmark C, et al. Cancer-associated fecal microbial markers in colorectal cancer detection. Int J Cancer. 2017;141(12):2528-2536. doi:10.1002/ijc.31011, 8. Ito M, Kanno S, Nosho K, Sukawa Y, Mitsuhashi K, Kurihara H, Igarashi H, Takahashi T, Tachibana M, Takahashi H, Yoshii S, Takenouchi T, Hasegawa T, Okita K, Hirata K, Maruyama R, Suzuki H, Imai K, Yamamoto H, Shinomura Y. Association of Fusobacterium nucleatum with clinical and molecular features in colorectal serrated pathway. Int J Cancer. 2015 Sep 15;137(6):1258-68. doi: 10.1002/ijc.29488. Epub 2015 Mar 13. PMID: 25703934. 9. Li YY, Ge QX, Cao J, et al. Association of Fusobacterium nucleatum infection with colorectal cancer in Chinese patients. World J Gastroenterol. 2016;22(11):3227-3233. doi:10.3748/wjg.v22.i11.3227. 10. Mima K, Cao Y, Chan AT, Qian ZR, Nowak JA, Masugi Y, et al. . Fusobacterium nucleatum in colorectal carcinoma tissue according to tumor location. Clin Transl Gastroenterol. (2016) 7:e200–e. 10.1038/ctg.2016.53. 11.Yamaoka, Y., Suehiro, Y., Hashimoto, S. et al. Fusobacterium nucleatum as a prognostic marker of colorectal cancer in a Japanese population. J Gastroenterol 53, 517–524 (2018). https://doi.org/10.1007/s00535-017-1382-6