Microbiome analysis of human breast cancer primary tumour tissue based on whole transcriptome analyses

Minarik G [1], Karaba M [2], Sedlackova T [3], Benca J [2,4], Repiska G [5], Krasnicanova L [5], Macuch J [2], Kalavska K [2,5] and Mego M [2,5]

[1] Medirex Group Academy, Nitra, Slovakia, [2] National Cancer Institute, Bratislava, Slovakia; [3] Science park of Comenius University, Bratislava, Slovakia, [4] Department of Medicine, St. Elizabeth University, Bratislava, Slovakia; [5] Faculty of Medicine, Comenius University, Bratislava, Slovakia

e-mail: gabriel.minarik@medirex.sk

Background

In recent years it was found that various organisms are inhabiting different parts of the human body. Not only bacteria, but also other forms of life and viruses. As a matter of fact, every organ has its own composition of microbiota. It has been revealed that bacterial composition has an effect on various diseases including metabolic disorders, inflammatory and autoimmune diseases and allergies. Effects on cancer were found at multiple different body parts including the stomach, colon, liver, lung and skin. It was proposed that microbiome contributes to 16–18% off all malignancies. To summarize, the results of published works, it can be concluded that Proteobacteria and Firmicutes are the most repeatedly reported as dominant phyla in breast tissue. Their presence in the normal or breast cancer (BC) tissue was suggested to be a result of adaptation to the fatty acid environment and metabolism in the tissue. The origin of the breast microbiome is not entirely clear, but at least part of it might be a result of translocation from the gastrointestinal tract, in addition to the skin, via the nipple-areolar orifices, nipple-oral contact via lactation and/or sexual contact. Multiple studies have been reporting differences in microbial compositions between the normal breast tissue and tumour tissue of breast cancer patients. A higher abundance of bacteria was found in healthy tissue compared to tumour tissue. However, there are some differences in the results of different studies. Normal tissue paired to tumour tissue was found to have different microbial composition compared to normal tissue of healthy women. Interestingly, similar composition of microbes in tumour sites compared to normal paired tissue were reported. Some studies also report, that compositions of normal adjacent tissue microbiome from women with benign tumours were closer to normal adjacent tissue of women with cancerous tumours than for tissue from healthy subjects. A significant finding is also, that they did not find different microbial profile dependent on stage of tumour or severity/invasiveness of disease. It is still being discussed how can possibly microorganisms influence breast cancer progress (if they can). Effects on immune system was proposed as a possible mechanism. Microbes can probably also alter some specific pathways and can induce DNA changes. In addition to the effects on disease itself, the microbiome has also the ability to influence treatment procedure.

Study aim

Since microbial reads can be found in standard Illumina RNA-sequencing of breast tissue, we used this fact to uncover microbial and viral composition of primary tumour tissue of breast cancer patients and breast tissue of healthy women. In our study we were looking for changes in the microbiome between healthy and cancer tissues and also between different phenotypes of disease: the presence of circulating tumour cells (CTC) in patient's blood, molecular subtypes of disease and multiple markers presence or absence.

Results

Microbiome in Primary Breast Tumour and Normal Breast Tissue

In breast tumour tissues of Slovak women (18 samples) the same phyla as in healthy tissue are the most abundant, while a portion is changed: *Proteobacteria* (44%), *Actinobacteria* (16%), *Firmicutes* (9%) and *Bacteroidetes* (3%). (see Figure 1A). In normal breast tissue of healthy Slovak women (5 samples) we observed the presence of 4 predominant phyla of bacteria, led by *Proteobacteria* (47% of total bacteria), while *Bacteroidetes*, *Firmicutes* and *Actinobacteria* follow (equally 12%). *Hymenobacter* (7%) and *Sphingomonas* (5%) were the most abundant on the level of genus. (see Figure 1B). In healthy normal tissue of Slovak donors, numerous bacterial taxa were present in higher numbers compared to tumour tissue of Slovak women. Using LefSe tool for statistical analysis, we report multiple taxa which transcript numbers correlate with disease. The most significantly overrepresented genus in normal tissue was *Hymenobacter* (as it is shown in Figure 3 with other taxa called by LefSe). In addition, *Bacteroides*, *Peanibacillus*, *Bifidobacterium*, *Pantoea*, *Collinsella*, *Sphingomonas*, *Methylobacterium* and multiple other taxa were called by LefSe. The most overrepresented of all taxa were transcripts of phylum *Bacteroidetes*. In contrast to many taxa abundant in normal tissue, we identified just a few candidates for overrepresented bacterial transcripts in tumour tissue. The most serious candidate would be *Streptomyces*, additionally there were overrepresented viruses *Siphoviridea* and in patients, genus *Acinetobacter*, *Rhodobacter*, *Micrococcus*, order *Corynebacteriales* and species *Priestia megaterium* were enriched in breast tumours. Results are shown in Figure 2 (Filtered more strictly for the purpose of visualization).

Associations between Clinico-Pathological Characteristics and Microbiome

CTC presence: We looked if there are changes in microbial transcript amounts between patients with circulating tumour cells in their blood and without them. Interestingly there were moregroups abundant in tumours of patients with circulating tumour cells (CTC) present in their blood (CTC+). Patients without CTC in their blood (CTC−) were enriched on bacteria species *Pasteurella multocida* and *Asticcacaulis excentricus* and genus *Delftia*. For CTC+ samples, the most abundant transcripts had order *Micrococcales*, genus *Rhodococcus*, *Bacillus*, *Devosia* and *Moraxella* (*Moraxella osloensis*). In addition, an abundance of family *Sphingomonadacea*, *Rhodobacteracea* and order *Rhizobiales* was observed. All taxa correlated with CTC status are shown in Figure 3A. It appears, that tumours of CTC+ patients might be richer for microbiome than tumours of CTC− patients. In tumours of CTC+ patients, 1.88-times more microbial transcript reads (compared to those marked as CTC−) were identified.

Hormone Receptor and HER2 Status: One of the cancer phenotypes comparison, where significant changes in microbial composition were observed was between HR+ and HR− disease. Those patients, which were positive on HR marker have been identified with multiple abundant taxa in their tumour tissues. Worth to mention are genus *Paracoccus*, *Actinomyces*, *Hydrogenophaga*, *Halomonas*, species *Cutibacterium granulosum*, *Bacillus cereus*, *Staphylococcus aureus*, *Clostridium tetani*, *Acinetobacter baumannii* and *Spirosoma Pollinicola*. HR- samples were possibly enriched for genus *Acinetobacter*, *Rhodobacter* and *Streptomyces*, family *Burkholderiaceae*, species *Priestia megaterium* (Figure 3B). In HER2+ tumours, bacterial group *Burkholderiales* were found to be overrepresented, although only 4 patients had HER2+ disease status (Figure 3C).

P53 Status:For patients positive for p53 protein, some taxa were underrepresented (*Sphingomonas*, Rhizobiaceae and species of *Staphylococcus*). Enriched was species *Klebsiella pneumoniae*.

T and N Stage, Tumour Grading: Phyla *Bacteroidetes*, family *Bifidobacteriaceae* (genus *Bifidobacterium*) and bacteria *Clostridium tetani* was associated with smaller tumours (in comparison to tumours smaller than 2 cm and tumours bigger than 2 cm). *Acidobacteria* correlated with a higher T stage. In the case of axillary lymph node involvement, some microbes are correlated with NO status, which is a group with less advanced disease with a better prognosis. The most possibly enriched are families: *Bacilaceae* (species *Bacillus subtilis*), *Oxalobacteriacea*, *Microbacteriaceae*, *Rhizobiaceae*, *Nocardiaceae*, *Hymenobacteraceae* (genus *Hymenobacter*) and *Acetobacteraceae*. In the case of tumour grading, a low grade was associated with enrichment of species *Bacillus aureus*, *Staphyloccocus aureus*, *Actinomyces oris*, *Spirosoma Polinica* and also family *Acetobacteraceae*. The high grade was found to be more inhabited by families *Burkholderiaceae*, *Lachnospiraceae* and species *Pseudolysobacter antarcticus*.

Molecular Subtype: Molecular subtypes of breast cancer were assigned with enriched taxa too. For HER2+ BC, order *Burkholderiales* were enriched compared to other subtypes. From triple negative breast cancer (TNBC) subtype we had just three samples, which were rich for a family *Xanthomonadaceae*, genus *Caulobacter*, species *Janthinobacterium sp_LM6* and *Streptomycessp_WAC01529*. Luminal A subtype was enriched with family *Staphylococcaceae*, *Dysgonomonadaceae*, genus *Shewanella*, *Paracoccus* and species *Ilumatobacter coccineus* (found also enriched in some luminal B samples). Luminal B subtype was harbouring transcripts of species *Lawsonella clevelandensis*, order *Aquificales*, genus *Anoxybacillus* (found to be common also in Luminal A). For Luminal A and B subtypes, also species *Finegoldia magna* was specific and plentiful in the number of transcripts (compared to the rest of samples) (Figure 3D).

Proliferation index Ki67: Multiple taxa correlated with Ki67 proliferation index. Ki67 < 20% tumours were found to be enriched with genus *Halomonas*, *Moraxella*, *Staphylococcus*, *Clostridium* and *Actinomyces*. The most enriched species with promising enrichment profile were *Bacillus cereus* and *Clostridium tetani*. On the other side, Ki67 > 20% tumours showed to be potentially more inhabited by *Mycetohabitans*, *Asticcacaulis* and *Sphingomonas*. Most promising looks *Mycetohabitans rhizoxinica*, *Rhodobacter sp*. and *Asticcacaulis excentricus* (Figure 3E).

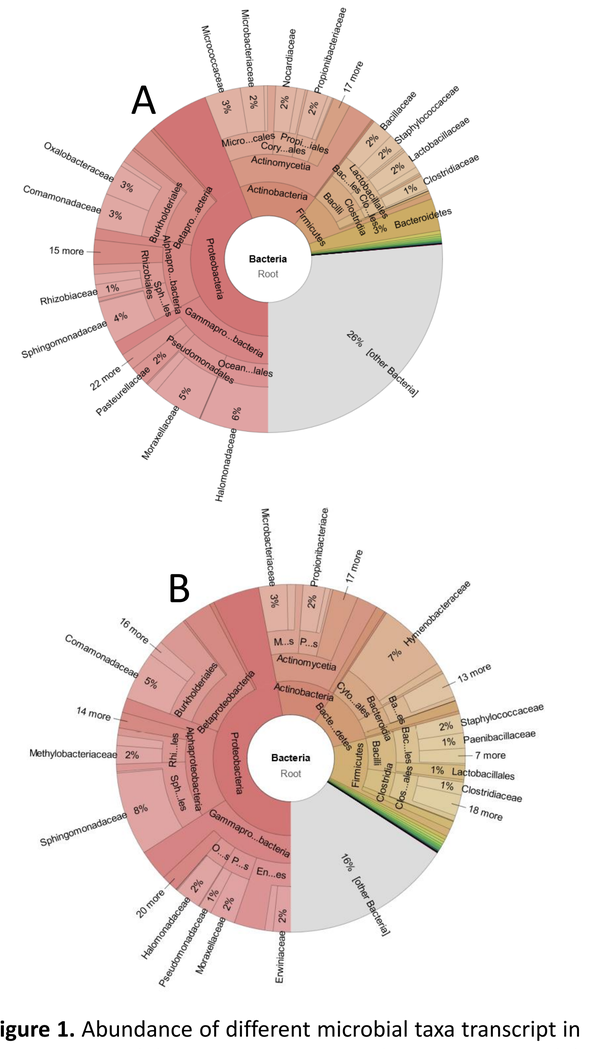

Figure 1. Abundance of different microbial taxa transcript in RNA-seq data. (A) in primary tumours of Slovak patients; (B) in normal breast tissue of Slovak cancer-free donors.

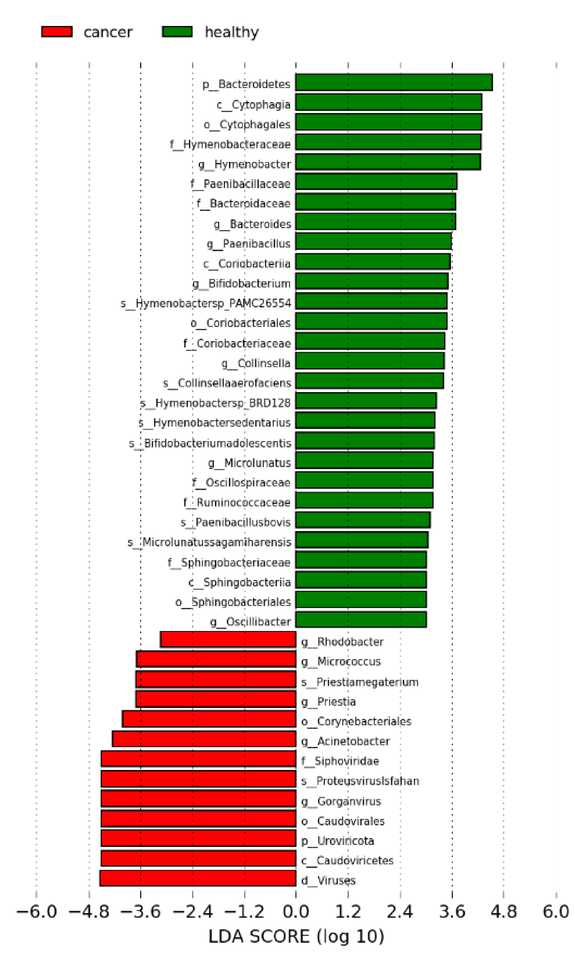

Figure 2. Differentially represented taxa (by different transcript numbers) between normal breast tissue samples (from cancer-free donors) and breast tumour tissue. Taxa on the left have a higher abundance of their transcript in primary breast tumour tissue, while those on the right have higher numbers in normal breast tissue.

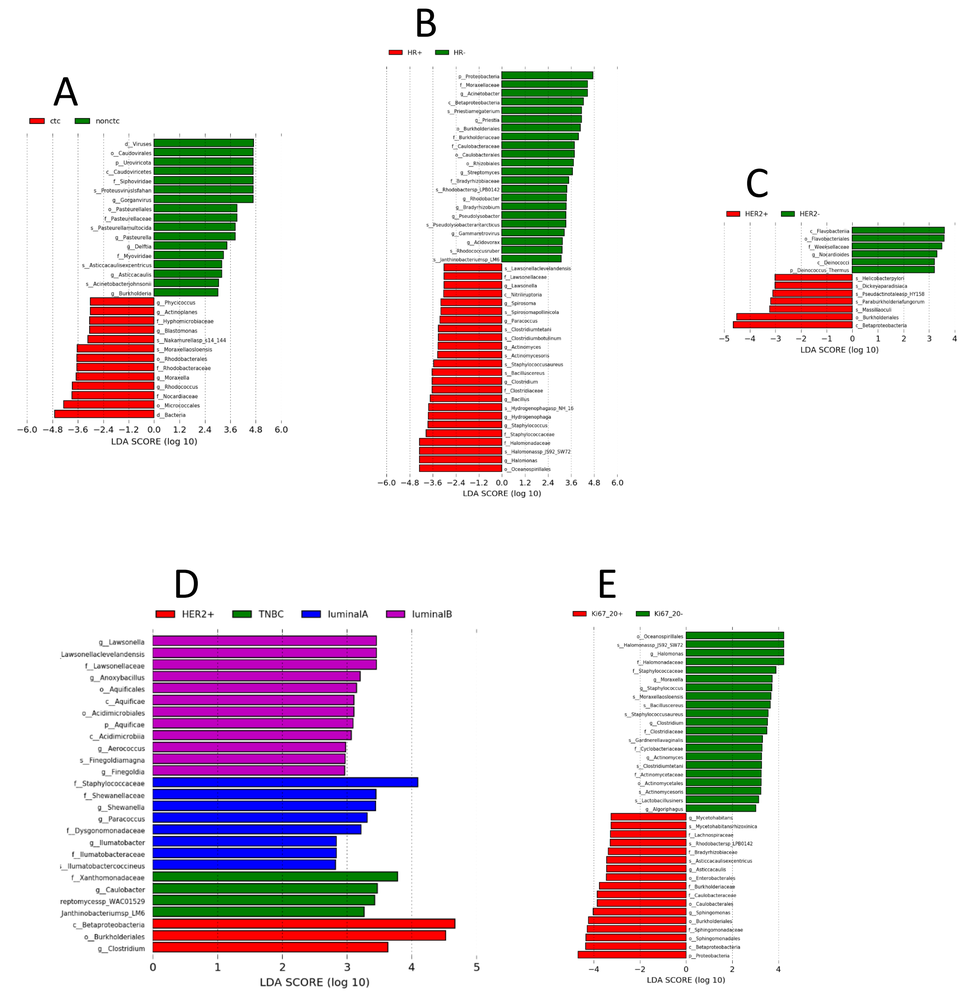

Figure 3. Differentially represented taxa in primary tumour tissues of Slovak patients between multiple markers statuses. (A) Comparison of the microbiome in primary tumours of patients with and without CTC detected in blood; (B) in primary tumours of patients positive on HR marker and negative on HR marker; (C) in primary tumours of patients positive on HER2 marker and negative on HER2 marker; (D) of molecular subtypes: Luminal A, B, HER2+, Triple negative; (E) in primary tumours of Ki67 > 20% and Ki67 < 20%.

Conclusions

In this study, we inspected the microbial composition of normal breast tissue and tumour tissue of the breast of donors from Slovakia. The most abundant phyla were in concordance with previous studies *Proteobacteria*, then *Firmicutes*, *Actinobacteria* and *Bacteroides*. Breast tumour tissue were different in microbial composition. Normal tissue appears to be richer for microbes, while many microbes were found to be overrepresented there. Differences in microbial compositions were also found when comparing molecular subtypes of disease, CTC status, markers (HR, HER2, p53), proliferation index *Ki67*, T and N stage of tumour and tumour grading. The reasons and biological relevance of microbial presence and amounts of their transcripts are not clear and additional studies will be needed to understand the influence on breast cancer and to exploit the microbiome for benefit of cancer patients.

Patients and Methods

The present study was approved by the Institutional Review Board (IRB) of the National Cancer Institute of Slovakia. Written informed consent was obtained from each participant prior to study enrollment. Whole transcriptome analysis (rRNA depleted RNA-Seq) of breast tumours and normal tissues (from cancer-free women) of 23 individuals from Slovakia was performed and different bioinformatic tools (Kraken2 and Metaphlan3) were used and compared to reveal differences in the microbial composition of analysed tissues and cohorts. Kraken2 has showed higher reliability for our data. Changes in microbiome profiles have been recorded not only between tumour and healthy tissues and different populations, but also analyzed for potential associations based on circulating tumour cells status and markers HR, HER2 and p53, proliferation index Ki67, T and N stage of tumour and tumour grading.

Reference

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