The microbiome in benign renal tissue and in renal cell carcinoma

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Research article

Keywords: kidney, microbiome, renal cell carcinoma, 16s DNA sequencing

Posted Date: July 8th, 2019

DOI: https://doi.org/10.21203/rs.2.10964/v1

Version of Record: A version of this preprint was published at Urologia Internationalis on November 12th, 2019. See the published version at https://doi.org/10.1159/000504029.

Abstract

Background To describe the renal microbiome and to determine differences of the renal microbiome in healthy and tumour-bearing parenchyma. Methods 10 biopsies from patients undergoing laparoscopic nephrectomy for renal carcinoma with no history of urinary tract infections within the last 6 months were included in this study. The identification of all microorganisms was done using 16s DNA sequencing. The beta diversity analysis was performed by Bray - Curtis dissimilarity. Results In all kidney samples, a plethora of microorganisms was found, with significant differences between benign and malignant renal tissue ($p< 0.0001$). Conclusions There is evidence that healthy kidney tissue as well as renal cell cancer tissue have a specific microbiome, thus opening new perspectives in renal physiology and tumour pathogenesis.

Background

The term microbiome is defined as the totality of all microorganisms of a habitat [1]. Due to recent developments in microgenetic and microbiologic analyses, the understanding of different microbiomes in the human body has increased. The mechanisms of bacterial growth and proliferation of a healthy tissue as compared to a tissue affected by disease, such as cancer, has not been explored so far.

Emerging evidence shows that there are multiple microorganisms inhabiting many sites of the body, including the urinary tract [1]. In the past, urine was thought to be sterile in healthy individuals. Recent studies show that there are a great number of microorganisms in the urinary tract [1] and changes in patients with type 2 diabetes mellitus [2], overactive bladder syndrome [3,4], urinary incontinence, interstitial cystitis [5], neuropathic bladder [6,7], sexually transmitted infections [8] and chronic prostatitis/chronic pelvic pain syndrome [9,10].

The aim of this pilot study was to examine the kidney microbiome. The second aim was to describe differences between healthy and tumour-bearing tissue, based on the hypothesis, that bacterial colonization of the kidney differs even within the very same organ, if parts are affected by disease.

Methods

This study was conducted with 10 formalin fixed paraffin embedded (FFPE) tissue samples of kidneys from patients who underwent laparoscopic nephrectomy because of renal carcinoma with no history of urinary tract infections within the last 6 months. Description of tumour entities and stage are recorded in Table 1. Samples were taken from the centre of the malignant tissue and from tumour free renal cortex. The testing of the whole genome with the identification of microorganisms was done using 16s DNA sequencing. DNA isolation was done with the GeneRead DNA FFPE Kit (Qiagen). DNA quantification was performed with the Qubit dsDNA BR assay kit using the Qubit 3.0 instrument (ThermoFisher) and quality control was performed with the standard genomic DNA analysis kit on the Fragment Analyser (Agilent Technologies). The library preparation was done with the QIAseq 16S / ITS Screening Panel. The Next

Generation Sequencing of the libraries was performed on MiSeqDx with V3 chemistry. The data was analysed with the CLC Genomics Workbench with the CLC Microbial Genomics module. For the supportive identification of the bacteria the COSMOSID database was utilized. The beta diversity analysis was performed by Bray - Curtis dissimilarity utilizing Principal Coordinate Analysis plot (PCo). The student´s t-test was used to determine the numerical difference of the microbiomes.

Results

Basic patient and tumour characteristics are given in Table 1. In the examined samples, a total number of 2.589.019 microorganisms could be identified. In healthy kidney tissue, 528.795 microorganisms (mean 105.759, median 141.014, SD 80.455,217) and in malignant tissue 2.060.224 microorganisms (mean 412.045, median 179.774, SD 438.586,893) were isolated. Numbers for each sample are shown in Table 1. A plethora of microorganisms was found, with significant differences between benign and malignant renal tissue ($p < 0.0001$) (Fig. 1).

We isolated 3 domains, 15 phyla, 16 classes, 19 orders, 27 families, 28 genera and 30 species of microorganisms. In the domain of the archeae we found two phyla, in the domain of the eukaryota 6 phyla, in the domain of the bacteria we found 7 phyla. The distribution of microorganisms shows differences in benign and malign tissue (Fig. 2).

The following microorganisms were found in healthy tissue only: Terrabacteria (phylum), Stenosarchaea (phylum), Microbacterium (genus), Pelomonas (genus), Staphylococcus (genus), Leuconostoc garlicum (species), Corynebacterium vitaeruminis (species), Anaerococcus nagyae (species), Ethanoligenens harbinense (species), Neisseria bacilliformis (species), Thermicanus aegyptius (species) and Leuconostoc mesenteroides (species).

Microorganisms that appeared in cancer tissue only were: Cyanophora paradoxa (species), Spirosoma navajo (species), Phaeocystis antarctica (species), Euglena mutabilis (species) and Mycoplasma vulturii (species).

Of the microorganisms found in both tissue types, the following were particularly frequent in cancer tissue ($p < 0.005$): Aeromonas salmonicida (species), Pseudoalteromonas haloplanktis (species), Parageobacillus toebii (species), Trachelomonas volvocinopsis (species), Mycoplasma mycoides (species) and Halomicrobium mukohataei
(species).

The Bray Curtis dissimilarity showed a clear cluster of the microbiome of the benign tissue in the PCo diagram (Fig. 3).

Discussion

This is the first attempt to describe the microbiome in renal tissue. Our study demonstrates that there is a microbiome in renal tissue and that there are differences in the microbiome of benign and malignant tissue. The major limitation is the small sample size as it was designed as a pilot study.

So far, healthy renal tissue was thought to be free of bacteria, although bacteria can enter the kidneys via the bloodstream [11-13]. The finding of bacteria in the kidney was always thought to be related to an infection, a microbiome of the kidney as such is unknown. The role of bacteria in the pathogenesis of kidney cancer is unclear.

However, an association between renal cell cancer and viruses has been published in several studies [14-17].

In the bladder, the association between Schistosoma haematobium infection and the development of squamous cell carcinoma by the endogenous synthesis of nitrosamines and oxygen radicals has been published [18, 19]. Xu [20] reported the association of the urinary microbiome (UM) with urothelial cell carcinoma (UCC) in a small number of patients. Pseudomonas and Anaerococcus were the most abundant genus in cancer urine samples. He suggested that urothelial carcinoma may be associated with altered microbiota of the urinary tract [20]. Another Chinese study from Wu [21] observed enrichment of Acinetobacter, Anaerococcus, and Sphingobacterium, and decrease of Serratia, Proteus, and Roseomonas in the cancer group when compared to non-cancer group. Cancer patients with high risk of recurrence and progression showed an enrichment of Herbaspirillum, Porphyrobacter and Bacteroides. Therefore, Beta diversity was significantly different in the cancer and non-cancer group. [21]. Concerning the influence of bacteria on cancer genesis, three Japanese studies evaluated the prophylactic effects of an oral Lactobacillus casei preparation in patients with superficial transitional cell carcinoma of the bladder. The results indicated that Lactobacillus casei strain Shirota could be effective for prevention and treatment of non-muscle-invasive bladder tumours [22-24].

We can support this research result. In our renal microbiome outcome, bacteria from the order of Lactobacilliales, from the family Leuconostocaceae were isolated to a higher extend in the healthy tissue of the kidney than in cancer tissue.

Another recent study demonstrated significant variations in microbial populations in prostatic secretions, voided urine, and seminal fluid from patients diagnosed with prostate cancer or benign prostatic hyperplasia. The group of patients with prostate cancer had a significantly higher number of microorganisms compared to the benign prostate hyperplasia (BPH) group, which differs from one another [25]. The Interaction of microorganisms and their hosts is complex. Molecular mechanisms are thought to be responsible for oncogenesis, tumour progression and response to anticancer therapy by changing the balance of host cell proliferation and cell death, influencing the immune system function and metabolism of host-produced factors and reaction to pharmaceuticals [26]. Therefore, diagnostic and therapeutic considerations concerning cancer and the microbiome require a multidisciplinary approach.

Conclusions

There is evidence that benign and malignant renal tissue have a specific microbiome, which differs from one another, opening a new field on renal function and tumour genesis.

Abbreviations

DNA deoxyribonucleic acid

FFPE formalin fixed paraffin embedded

PCo coordinate analysis plot

UM urinary microbiome

UCC urothelial cell carcinoma

BPH benign prostate hyperplasia

Declarations

Ethics approval and consent to participate

The Karl-Landsteiner-University of Lower Austria states in its policy regarding “Good scientific practice”, Version 1 from September 2015, under point 2.3 concerning measures not to be submitted to the ethics committee on humans:
No submission to the ethics committee is required for medical actions that are exclusively performed for the health benefits of an individual patient. That means, if the objective is not the gain of knowledge, such medical actions ("therapeutic attempt") are not to be considered research projects and therefore not "ethics commission" is needed. In our case, nephrectomy was performed in order to cure a renal cancer in each and every tissue sample utilized. Surgery had to be performed, the tissue was not destroyed postoperatively, but referred to us for further analysis after tumour histology was finished. This is in accordance to Art. 35, Declaration of Helsinki, 2008. A written informed consent for participation was given by all patients before nephrectomy during the inpatient admission.

Consent for publication

A written informed consent for publication was given by all patients before nephrectomy during the inpatient admission.

Availability of data and material

The datasets used and analysed during the current study are available from the corresponding author on request.

Competing interests

The authors declare that they have no competing interests.

Funding

Qiagen Company provided the analysis equipment for DNA isolation of microbes. Qiagen company has not contributed to this study in any other way, such as study design, collection and interpretation of data or the writing of the manuscript. This was performed solely by the authors.

No further funding or sponsoring was received for this study.

Authors' contributions

SH contributed the study concept and design, analyzed and interpreted data and wrote the manuscript. SM and LL were major contributors in the writing of the manuscript. CF performed the histological examination of the kidneys. All authors read and approved the final manuscript.

Acknowledgements Qiagen Company provided the analysis equipment.

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Tables

	sex	age	Tumor type	TNM	side	Numbers of microorganism
Patient 1	male	59	clear cell carcinoma	pT2b, NX, L0, V0, R0	left	Readcount normal tissue 141014
						Readcount tumor tissue **815374**
Patient 2	male	4676	chromophobic cell carcinoma	pT2a, NX, L0, V0, R0	left	Readcount normal tissue 142649
						Readcount tumor tissue **956600**
Patient 3	male	48	clear cell carcinoma	pT3a, NX, L0, V0, R0	right	Readcount normal tissue 19578
						Readcount tumor tissue **179774**
Patient 4	male	46	clear cell carcinoma	pT1b, NX, L0, V0, R0	left	Readcount normal tissue 201554
						Readcount tumor tissue 43800
Patient 5	male	49	clear cell carcinoma	pT1b, NX, L0, V0, R0	left	Readcount normal tissue 24000
						Readcount tumor tissue **64676**

Table 1: Basic patient and tumour characteristics with microorganism count

Figures

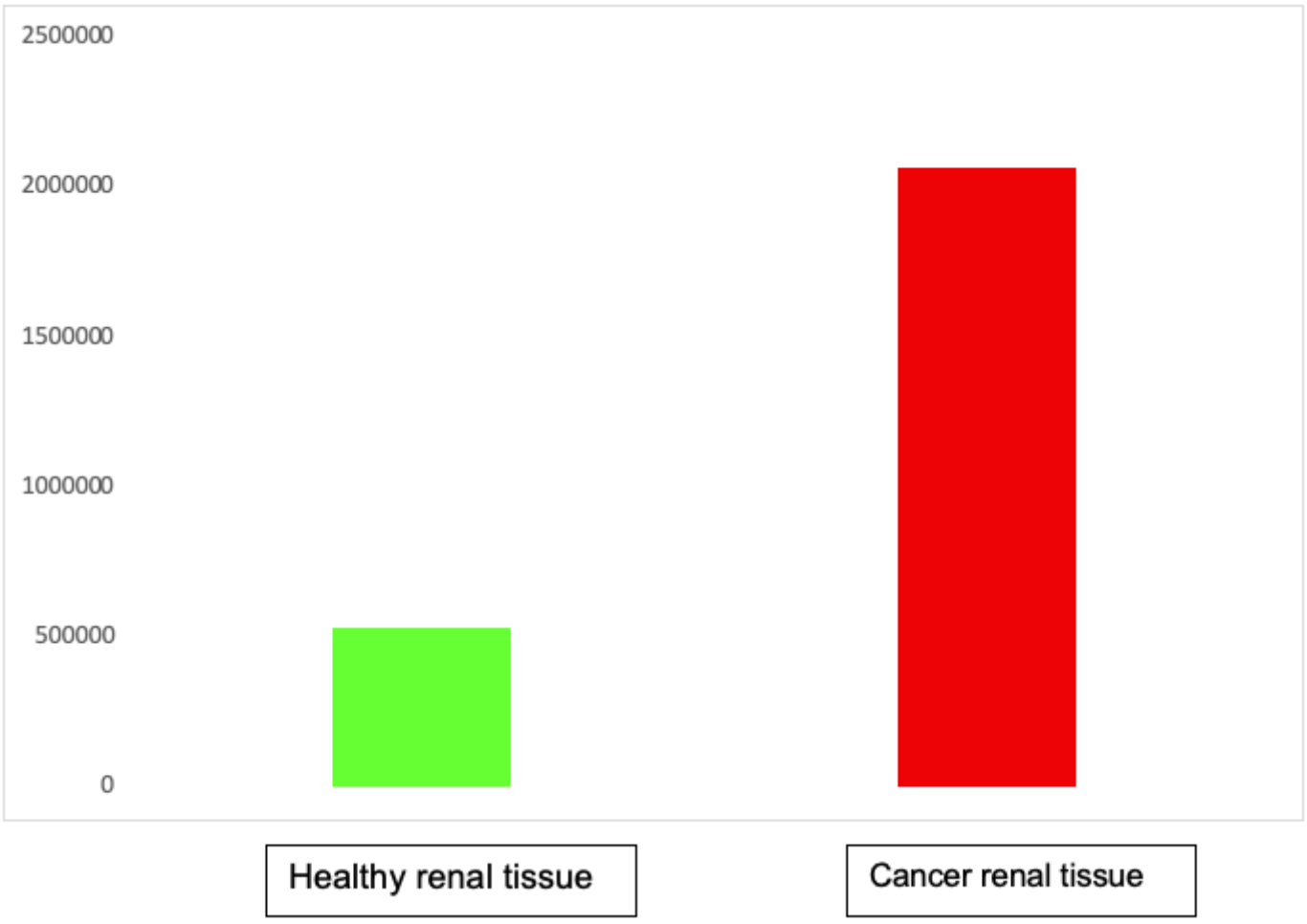

Figure 1

Total numbers of microorganisms in healthy and cancer tissue

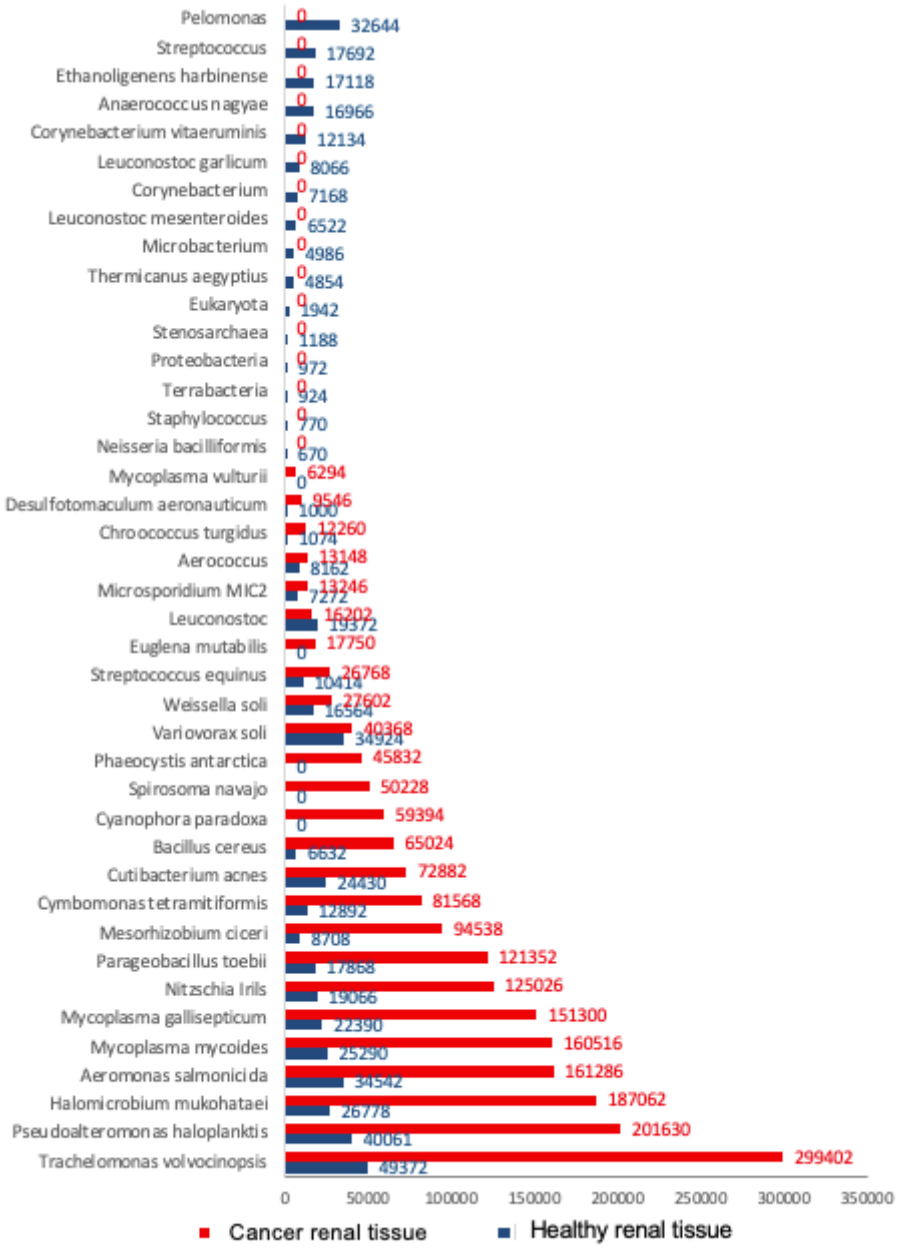

Figure 2

Total readcounts of indentified microorganisms

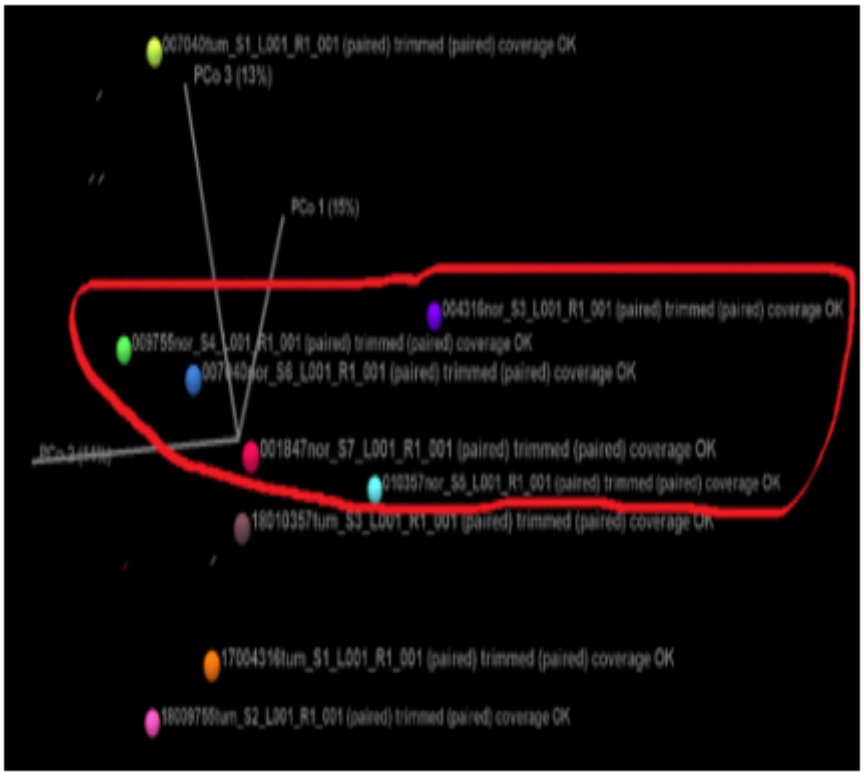

Figure 3

PCo plot (Bray-Curtis dissimilarity)