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The Carcinogenic Potential of Microbial Infections of the Prostate and the Role of Contaminations

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Abstract

Several studies have identified bacteria and viruses in human prostatic tissues. The tumor microenvironment of prostate carcinoma is a complex community of genetically transformed cancer cells, non-neoplastic cells, and a diverse collection of microorganisms. Each of these components may contribute to carcinogenesis; however, the role of the microbes is the least well known. A variety of detection techniques have been used, such as PCR-based approaches, fluorescence *in-situ* hybridization, immunological detection assays, and (bacterial) cultivation. Detection rates vary between these methods and each method has specific advantages and limitations. However, confounded by the high risk of contamination during or after the biopsy, it is challenging to make solid conclusions about the presence of certain microorganisms and its possible role in disease formation or progression. This doubt increases with the sensitive detection methods such as next generation sequencing technologies if there are no proper controls. Resident microbial communities often differ between healthy and diseased states, but whether these differences are of primary etiological importance or secondary to the altered inflammatory environment remains largely unknown.

Keywords: Prostate; Microbiome; Contamination

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Key Message

There have been studies showing the presence of the microbial infections in the prostate, but due to lack of proper controls and the risk of contaminations, a solid conclusion cannot be made.

Introduction

Worldwide, more than 670,000 men are diagnosed with prostate cancer (PCa) each year and 80,000 deaths are attributed to this cancer annually in Europe (http://info.cancerresearchuk.org and http://www.pcf.org), making it the most common non-cutaneous malignancy in men of Western countries. Furthermore, post mortem prostates exhibit high frequencies of PCa related abnormalities; it is estimated that around half of all men in their fifties and 80% of 80 year olds have histological evidence of this disease (http://info.cancerresearchuk.org). Almost all PCas (95%) are adenocarcinomas that originate in glandular tissue. The exact causes of PCa are unknown; the high frequency of PCa points to common causative circumstances and/or agents. Several risk factors have been identified, such as age, ethnicity, genetic predisposition, and diet [1,2]. In addition, many studies have presented circumstantial evidence that chronic inflammation of the prostate is an important contributing factor for prostate carcinogenesis [3,4]. Inflammatory mechanisms could stimulate carcinogenesis by causing DNA damage, promoting cellular turnover and creating a tissue microenvironment that enhances cell proliferation, migration and angiogenesis. In accordance with this hypothesis, daily use of anti-inflammatory medicines such as aspirin and ibuprofen decrease the risk of PCa [5].

Literature Review

The human body is home to an extraordinary diversity of microbes, which are increasingly suggested to have pivotal roles in human health. Human microbiome sequencing projects have revealed intriguing correlations between specific patterns of microbial diversity and multiple aspects of host health, including autoimmune disorders, diabetes, and obesity [6,7]. However, Microbial infections are known to trigger inflammation; therefore, infections of the prostate may increase the likelihood of PCa development and/or may enhance its progression [6,7]. Several

studies have been carried out to search for viruses and bacteria in diseased prostates. Such studies fall into two categories: global analyses using broad/multiple detection methods to assess the prevalence of numerous species and studies that seek to specifically identify a single pathogen (or a limited number of species). The investigations that fall into the second category focused on microorganisms with known pathogenic properties, in particular sexually transmitted infectious agents, including the causative agents of syphilis, *Treponema pallidum*; and gonorrhea, *Neisseria gonorrhoeae*. Other examples of microorganisms that have been investigated are those found in cases of urinary tract infections or prostatitis, such as *Escherichia coli, Klebsiella pneumonia,* **Pseudomonas aeruginosa,** *C. trachomatis*, *Ureaplasma urealyticum, Mycoplasma hominis* and the protozoan *Trichomonas vaginalis*. In addition, viruses with suspected or known carcinogenic potential, such as HPV, CMV and EBV have been investigated. Generally speaking, most of these studies failed to find a significant association between a particular microorganism and PCa. Due to differences in detection methodology, sampling, origin and numbers of samples and (lack of) controls, these studies were often contradictory or inconclusive. Several bacterial species have been found in the prostate, including *Escherichia, Chlamydia, Neisseria, Staphylococcus* [6,7]. Recently, *Propionibacterium acnes (P. acnes)* has been detected in prostate tissue from patients with prostatitis and prostate cancer [8-10]. Since several decades, an explosion of descriptive analyses such as MetaHIT project and the Human Microbiome Project (HMP), have begun to delineate the human microbiome. Residents of nasal fluids, intestinal tract, skin, oral cavities and urogenital tract have been identified using next generation sequencing, cultivation and different omics methods [11]. Traditionally, microbial genome sequencing was restricted to a small number of species that could be cultivated. Recently, the rapid development of culture-independent methods has allowed the researchers to sequence microbial communities directly from environmental samples. This approach is commonly referred to as 16S rRNA sequencing or "metagenomics" [12,13]. On the other hand, however, DNA contamination is ubiquitous in laboratory equipment commonly used to analyze the microbes that inhabit the human body. This contamination could seriously undermine cutting-edge work to understand the 'microbiome' [14]. However, one potential confounder of these sequence-based approaches is the presence of contamination in DNA extraction kits and other laboratory reagents [15,16]. Here we summarize current knowledge concerning the link between bacterial (and viral) infections and prostate pathologies. We discuss the identification of microbes in diseased prostates and the complications associated with obtaining such information; the inflammatory potential of these bacterial/viral agents, the quest for a microbial etiology for PCa, and the link between inflammation and PCa initiation and progression. In addition, current aspects of prostate cancer research such as the role of androgen signaling, epigenomics and the search for the PCa 'cell of origin' are discussed in the context of an infection scenario.

Detection of Microorganism in Diseased Prostates

General considerations

Several issues must be considered in order to accurately investigate and evaluate the presence of microorganisms in human prostates. It is obvious that the sample collection process and choice of detection method critically influence outcome. First, there is a risk of viral and bacterial contamination of samples from external sources. Contamination can potentially be introduced during sample collection or after resection if samples are stored under non-sterile conditions. Indeed, it is challenging to collect human prostate tissue samples under completely sterile conditions [17,18]. Biopsy samples are collected with a biopsy needle that needs to traverse a typically 'contaminated' surface, the rectum. Alternatively, a prostate biopsy can be obtained trans-urethral; however, once again a potentially 'contaminated' surface, the urethra, must be traversed. To introduce some contamination control to these procedures, biopsy needles and the relevant human body surfaces are thoroughly disinfected before sample collection. In the case of samples taken by radical prostatectomy it has to be considered that essentially all men who undergo radical prostatectomy would have previously had a trans-rectal biopsy. Thus, in this scenario one must consider the possibility that bacterial or viral contamination of the prostate sample could have been introduced during an earlier procedure. Proper biological controls are essential to evaluate the presence of microorganisms in prostate tissues. Ideally, such controls should be tissue samples from healthy prostates. However, access to samples from truly healthy prostates is rare as typically men undergoing a needle biopsy have raised PSA levels and show additional signs of disease. Therefore, samples from normal prostates are usually only obtained from autopsy. In addition, non-cancerous samples from tissues adjacent to prostate tumors might be useful to analyze. However, due to the close vicinity to the tumor site, the prevalence of bacteria and viruses in such samples are usually not conclusive regarding the role and involvement of these microorganisms in disease formation.

Methodologies

A further consideration must be the choice of methodology used to detect microorganisms in the human prostate. A variety of techniques are available; PCR-based approaches, fluorescence *in situ* hybridization (FISH), immunological detection assays, and (bacterial) cultivation. Detection rates vary between these methods and each method has specific advantages and limitations. PCR-based techniques are frequently used to either amplify bacterial 16S rRNA and/or organism-specific sequences. The advantages of such an approach are high sensitivity and independence from cultivation of the isolated microorganism. However, the high sensitivity can in itself be problematic in some cases. Moreover, it has to be taken into consideration that this method does not distinguish dead/non-viable from alive microbes. In addition, this method relies on the accessibility of DNA; as bacteria differ in their cell wall composition and outer

membrane structure, and lysing agents such as lysozyme and proteinase K (two enzymes frequently used in commercial nucleic acid extraction kits) exhibit microbe-specific differences in lysis efficiencies. Thus, proper conditions for bacterial DNA extraction from tissue samples must be established. Immunological detection methods such as immunohistochemistry (IHC) or immunofluorescence (IF) typically visualize surface structure components of bacteria or viruses. These methods depend on the availability of highly specific antibodies capable of recognizing a bacterial/viral antigen; the selected antibody must not cross-react with antigens of other microbe species or host tissue. An advantage to this method is its ability to pinpoint the exact location of microorganisms within tissue samples. With the introduction of, tissue microarrays, capable of displaying hundreds of samples on a single glass slide IHC/IF has become more powerful as a large number of samples can be tested in a single experiment. A closely related technique, fluorescence *in situ* hybridization (FISH), can also visualize the location of microorganisms within prostate tissue samples. It is usually more specific than antibody-antigen-recognition approaches because the labeled FISH probe hybridizes with DNA specific for the detected organism. However, the bacterial or viral DNA must be accessible for hybridization, i.e. the labeled oligonucleotide probe has to fully penetrate the tissue as well as the bacterium/virus. An indirect approach is serological analysis; with this method, serum antibodies directed against a specific microorganism are detected; quantification of the antibody titer is usually achieved by enzyme-linked immunosorbent assay (ELISA), complement fixation test (CFT) or indirect IF. These methods require knowledge about the immunoreactive factors (and ideally of their epitopes) of the target organism, i.e. its cell surface antigens. Commercial kits are available that detect, for example, serum antibodies to *Chlamydia trachomatis* (*C. trachomatis*), herpes simplex virus (HSV), human cytomegalovirus (CMV), *Neisseria gonorrhoeae* (*N. gonorrhoeae*) and *Treponema pallidum* (*T. pallidum*). A major drawback to this method is that it is not possible to differentiate between active and past infections at the time of sampling. Moreover, infectious agents have evolved strategies to circumvent or manipulate the adaptive immune response, e.g. via phase-variable surface proteins. The traditional technique used for the detection of bacteria in prostates is bacterial cultivation, using solid or liquid media to propagate the organisms. This method detects viable bacteria; cultivated bacterial colonies on agar plates can then be further analyzed, e.g. using PCR/sequence identification. However, some potential drawbacks to this technique should be mentioned: not all microbes are culturable under (standard) laboratory conditions; some bacteria have specific requirements regarding cultivation conditions, such as medium composition, oxygen tension and cultivation temperature and growth time. Other issues are also of importance: it is unlikely that infectious agents are evenly distributed in the (diseased) prostate; thus, the investigation of only one tissue sample per patient might not be sufficient to detect microorganisms. Indeed, Sfanos et al. [4,17] showed that detection rates were highly dependent on the number of samples taken per patient: When only one biopsy needle core was investigated, 50% of patients were PCR positive for 16S rRNA. However, when investigating three cores per patient the detection rate increased to 87%. In summary, an ideal study comprises more than one detection method and multiple, appropriate controls to investigate the prevalence of microorganisms in (diseased) prostates.

Quest for causality

Establishing a firm biological link between a pathogen and oncogenesis is challenging – indeed the microorganism might have long been eliminated before tumor growth is detected. Furthermore, it is likely that there are numerous other confounding factors that impact on possible pathogen-related oncogenesis, for example, despite the carcinogenic effect of *Helicobacter pylori* infections, it is clear that only a very small fraction of infected individuals develops gastric cancers during their lifetime. Despite these complicating factors some general criteria to draw a link between an infectious agent and PCa formation can be formulated:

1. A positive association between the presence of the microorganism and the development of PCa; epidemiological studies should identify the agent as a major risk factor.
2. A model system (tissue culture model or animal model) should support the assumption that the infectious agent has a crucial impact on host (cell) fate (e.g. anti-apoptotic properties, induction of cellular transformation).
3. Protection (e.g. antibiotic treatment, immunization) against the microorganism should lower the cancer incidence.

Besides the direct carcinogenic activity of an infectious agent, other contributions are also possible, and in the case of PCa might be considered more likely, e.g. a role as an inflammatory agent. In an extension to this, it could be envisaged that after tumor development an infectious agent might support the progression of PCa and its metastasis, e.g. by activating angiogenic factors and/or the plasminogen system. Naturally, one must also always consider that the infection itself is a secondary consequence of tumor formation, due to altered host homeostasis and factors such as immunosuppression, altered nutrient availability, tissue trauma and a hypoxic microenvironment that could be favorable for the invading microbe. Very little data are available concerning the existence of such microbial invaders that take advantage of the altered microenvironment. They could have a possible active role as inflammation-inducing agents or can they be considered simply as passive bystanders. Additionally, an invading microbe could have a commensal relationship with its host, with no dependence on disease state of the prostate and with no impact on disease initiation or progression. However, the existence of a general prostatic bacterial flora is unlikely, since no ubiquitously distributed microorganism has been detected so far [4,18].

Detected microorganisms

Many studies have been carried out to search for viruses and bacteria in diseased prostates. For reviews of these studies we refer readers to: Sutcliffe; Chang and Pasonne; Klein,

Silverman [4,18,19]. Such studies fall into two categories: global analyses using broad/multiple detection methods to assess the prevalence of numerous species and studies that seek to specifically identify a single pathogen (or a limited number of species). The investigations that fall into the second category focused on microorganisms with known pathogenic properties, in particular sexually transmitted infectious agents, including the causative agents of: syphilis, *Treponema pallidum*; and gonorrhea, *Neisseria gonorrhoeae*. Other examples of microorganisms that have been investigated are those found in cases of urinary tract infections or prostatitis, such as *Escherichia coli, Klebsiella pneumonia,* **Pseudomonas aeruginosa,** *C. trachomatis*, *Ureaplasma urealyticum, Mycoplasma hominis* and the protozoan *Trichomonas vaginalis*.

Discussion

In addition, viruses with suspected or known carcinogenic potential, such as human papillomavirus (HPV), Human cytomegalovirus (CMW) and Epstein-Barr-Virus (EBV) have been investigated. Generally speaking, most of these studies failed to find significant associations between a particular microorganism and PCa. Due to differences in detection methodology, sampling, origin and numbers of samples and (lack of) controls, these studies were often contradictory or inconclusive. Rather than discussing the details of the individual case studies themselves we will focus our discussion on two infectious agents that are of particular interest for their potential association with prostate pathologies, namely the bacterium *Propionibacterium acnes* and the virus XMRV. The global approach previously mentioned has resulted in broader studies, which are not designed to detect a specific microbe but hope to identify any possible invader present in diseased prostates. A good example of this approach is the study by Sfanos et al. in 2007 in which 170 samples from 30 prostate cancer patients were subjected to 16S rDNA amplification Bacterial 16S rDNA could be amplified from 37% of samples tested. In 87% of all patients bacterial DNA from one or more species could be detected. In total, 83 distinct microorganisms were identified. The most frequently observed bacteria were: *Acinetobacter spp* (10 out of 30 patients), *Escherichia spp* (10/30), *Pseudomonas spp* (8/30), *Methylophilus spp* (8/30) and *Streptococcus spp* (6/20). Interestingly, by applying microbe-specific PCR, infectious agents that had been suspected as having a role in PCa, such as *C. trachomatis*, HPV and XMRV, were not detected (or were detected at low frequencies) in this investigation.

Conclusion

Based on the evidence and the results of my colleagues, the author of this study concluded that there is no significant association between the presence of a particular microbial species and histologic evidence of acute or chronic inflammation of the prostate. In conclusion, we would like to emphasize the challenging nature of performing scientifically meaningful studies in this field. One possible future strategy would be a collaborative effort of several laboratories in order to test the validity of different detection methods and in which there was free exchange of protocols, samples and reagents. To date, there are no data that convincingly demonstrate a link between PCa and a particular microbe. However, if one excludes the possibility of contamination, many different bacteria and viruses have been detected in a high portion of diseased prostates, indicating that prostate infections occur frequently, if assume those are not contaminations. Such infections could reasonably be predicated to augment the inflammatory microenvironment, thus contributing indirectly to PCa formation or progression.

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