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Research Article

Immunotherapy of Prostate Cancer Patients could Overexpress The Virulence Factor Genes of *E.faecalis*

Tayebe Talebzade[1], Fahimeh Baghbani-arani[2], Soha Sadeghi[3], Arvin Haghighatfard[4]*, Niloofar Ahmadi[5], Massoud Houshmand[6] and Ali Dezhgir[4]

Abstract

Prostate cancer is the most prevalent and second cause of death from cancer in men worldwide. Immunotherapy is a new method for the treatment of several cancers that fights cancer cells by strengthening the immune system through some medications. While immunotherapy is a useful method for cancer treatment; its' side effects still are not totally clarified. Numbers of prostate cancer patients which take immunotherapy are experiencing prostate inflammation and prostatitis after treatment period. *Enterococcus faecalis* is Gram-positive and catalase-negative cocci that are common in the intestines of humans and other animals and cause most enterococcal infections such as intestinal infections, prostatitis, gastroenteritis and endocarditic. Present study aimed to evaluate the mRNA level of virulence genes which are involved in *Enterococcus faecalis* pathogenesis in prostate cancer patients that treated by immunotherapy.

Expression level of gelatinase E (*gelE*) and Enterococcal surface protein (*esp*) genes were examined by Real time PCR in three groups of 68 male subjects. Group A normal subjects, group B prostate cancer patients before start treatment and group C prostate cancer patients after six months immunotherapy period.

Results were showed significant (P<0.05) over expression of both genes (*gelE* and *esp*) in group C against the group B. According to the results, it is reasonable that immunotherapy may have side effects such as increasing the pathogenicity risk of microflora in patients. Maybe these side effects could cause further infections after ending the immunotherapy of cancer. Antibiotic usage after or at the same time of immunotherapy period could prevent possible infections of microflora including *E.faecalis*.

Keywords: Prostate cancer; Immunotherapy; Side effects; *Enterococcus faecalis*

Introduction

Immunotherapy is a new method for the treatment of some cancers and allergic diseases that by strengthening the immune system through some medications fights cancer cells. Rosenberg and his colleagues in the National Institutes of Health in the United States brought in the treatment of cancer by immunotherapy for the first time. Immunotherapy could categorize into two groups, increase or decrease the immune response [1].

Immunotherapy of prostate cancer was approved by FDA in 2010 [2]. Several immunotherapeutic approach were examined in clinical trials of prostate patients including checkpoint inhibitors, oncolytic virus therapies, therapeutic vaccines, adoptive cell therapies and adjuvant immunotherapies [3].

One of the most prevalent side effects of prostate cancer therapy is the post treatment infections in patients which is due to therapies" modulations on pathogenicity of *E.Coli* and *Enterococcus faecalis* [4]. Enterococci are Gram-positive and catalase-negative cocci that are common in the intestines of humans and other animals.

Enterococcus faecalis have caused the most enterococal infections in human instance endocarditic, gastroenteritis and intestinal infections [5]. Several regulatory proteins were identified as pathogenicity factors in clinical and commensal Enterococcus isolated. Nevertheless Enterococcus virulence mechanisms as opportunistic pathogens still are not completely known. A useful method to investigate the virulence of microorganisms is the gene expression study [6].

Affiliation:
[1]*Department of Microbiology, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran*
[2]*Department of Genetics & Biotechnology, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran*
[3]*Department of Cellular and Molecular Biology, Nour Danesh Institute of Higher Education, Isfahan, Iran*
[4]*Department of Biology, Tehran North branch, Islamic Azad University, Tehran, Iran*
[5]*Department of Microbiology, science and research branch, Islamic Azad University, Tehran, Iran*
[6]*Department of Medical Genetics, National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran*

***Corresponding author:**
Arvin Haghighatfard,
Department of Biology, Tehran North branch, Islamic Azad University, Tehran, Iran,
Tel: +98 21 44787390, Fax: +98 21 44787399
E-mail: Arvinland@yahoo.com

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GelE (gelatinase E) is a metallo-endopeptidase extracellular protein that has a significant role in virulence and extending of bacteria in its own host. *ESP* (enterococcal surface protein) is an enzyme that helps *E.faecalis* to evade the host immune system [7]. The surface protein of Enterococcus is encoded by *esp* gene that associated with increasing virulence, colonization, stability and biofilm formation in urinary tract [8]. Previous studies in animal models were shown the role of surface protein of *E. faecalis* in colonization of bacteria and survival of urinary tract infections [8,9]. Over expression of these genes might increase the *E. faecalis* infections as well as infections caused by antimicrobial resistance [5].

The aim of this study was evaluating the side effects of immunotherapy on pathogenicity potency of *Enterococcus faecalis* in prostate cancer patients by using methods of pathogenicity gene expression study.

Materials and Methods

Subject selections and stool sampling

At the first level 173 early diagnosed prostate cancer patients from two hospitals of Tehran and 173 related normal subjects were included to the study. Patient group and group of normal subjects have been matched in age range, BMI and socioeconomic situation. None of the subjects had current or history of sever medical condition including any infection or allergy, drug abuse or alcohol dependence. The Gleason grading system was used to stage the prostate cancer subjects. Gleason scale is used to evaluate the prostate cancer stages based on microscopic appearance of prostate biopsy. Gleason scores range from 2 to 10, with higher number indicating greater risks and higher mortality. In addition ABCD rating was used to analyze the cancer stage right after the patients' admission. ABCD rating is a staging system for prostate cancer which uses the letters A, B, C, and D. "A" and "B" is indicating that cancer is confined to the prostate "C" indicates that cancer has grown out of the prostate but has not spread to lymph nodes or other tissues. "D" refers that cancer has spread to lymph nodes or other tissues and may lead to metastasis.

Present study was done on 68 patients of 173 early diagnosed prostate cancer patients that were started immunotherapy as first therapy approach *and E.faecalis* was successfully isolated from their samples along with 68 related normal. As microbial flora is strongly related to life style of individuals, normal subjects had selected from related individuals which were lived with patients. Stool sampling operated just day before starting treatment and day after treatment period has completed. All patients were treated by Adoptive T cell therapy method for six months. All subjects participated in a meeting to explain the aim and steps of study. No interference in patients' treatment process was conducted. Written informed consent had been provided for all subjects. Central ethical committee of young researchers and elites club has approved the study.

Identification of Enterococcus faecalis

The sampling of stool from subjects in the three mentioned groups was as follows: At first, a few grams of fecal was added into azide dexterous broth medium in order to isolate Enterococcus from stool, then re-cultivation was performed in the general agar medium after 24 hours of incubating.

Generic colonies were determined to the genus and species level in according to their membranes by Gram-stain, catalase test, the potency to bear 6.5% NaCl and biochemical tests [10]. The isolates identified as Enterococcus were used in this study and species identification was confirmed by performing Real-Time PCR to detect the $ddl_{E.faecalis}$ gene in *E.faecalis* (this gene encode D-Ala:D-Ala Ligases and is a specific marker gene for *E.faecalis* detection) [11] (Figure 1).

Gene expression study by real time PCR

Total RNA was extracted from stool sample's colonies of *E.faecalis* according to standard protocols of RNA Purification kit (GeneJET™ RNA purification Kit#K0732, Thermo Scientific, Latvia). The cDNA was synthesized by using a transcription first strand cDNA synthesis kit (RevertAid premium first strand cDNA synthesis kit #K1652, Thermo Scientific, Latvia) according to manufacturer's protocol, also quantitative RT-PCR was performed by applied CFX96 Touch Real-Time PCR detection system (BIO-RAD, California, United States) and SYBR green kit (Thermo Scientific Maxima SYBR Green/ROX qPCR master mix (2X) #K0221, thermo scientific, Latvia) according to manufacturer's protocol. Normalization of gene expression analysis had been operated by using 16srRNA as housekeeping gene. Primers for *gelE* and *esp* as target genes and *16srRNA* as reference gene designed by "oligo7" software and blasted on NCBI website for scrutinizing of specificity. Primers were presented in Table 1.

Statistical Analysis

Descriptive data was presented as mean ± SD (range) and level of statistical significance was set at $P < 0.05$. First normal distribution for continuous variables was estimated by the Kolmogorov-Smirnov test. Then individual and paired t tests were applied for gene expression alterations analysis.

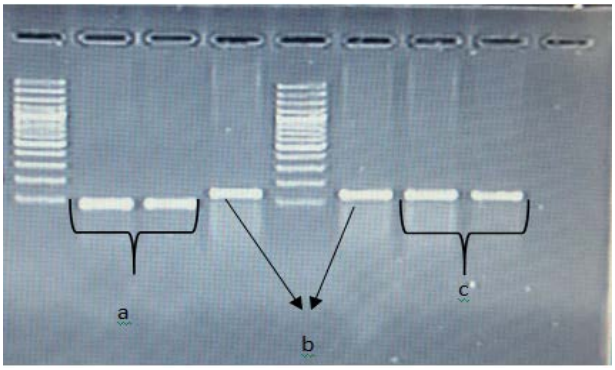

Figure 1: Gel electrophoresis (Agarose 1%) for Real Time PCR primers of two target genes and one housekeeping gene. a: *ESP* 100bp, b: *GelE* 129bp, c: *rRNA-16S ribosomal RNA* 127bp, lader 100bp

Results

Clinical results

The mean age of all of the individuals in this study was 69 ± 8.5 yrs. Prostate-Specific Antigen (PSA) level mean was 19.6 ± 8.5 as reported by PSA test result. The patients were divided into two groups in according with stage (ABCD) of disease in starting time of treatment: 38 patients with stage B and 30 patients with stage C. A 'Gleason' score was given after pathology report as follow: 37 patients with 6GS, 16 patients with 7GS and 15 patients with 8GS (Table 2). 13 of 30 patients with stage C and 11 of 38 patients with stage B returned to the clinic with pain and inflammation of the prostate a few months after immunotherapy treatment.

Gene expression results

Results of the study were showed that a significant over expression in *gelE* (P=0.02, Mean ratio=1.33), *esp* (P=0.03, Mean ratio =1.36) genes were revealed in "after treatments" group in comparison to before treatment and normal subjects (Table 3). No significant difference was found in expression of these two important virulence factor genes between before treatments patients isolates and isolates from their related normal individuals (P>0.05, Mean ratio≈10) (Table 3).

Discussion

Cancer immunotherapy tries to excite the immune system in order to destroy tumors. The most important advantages of immunotherapy compared with other cancer therapy methods including specific effects and less hitting to non-cancer cells. Although several researches were clarified the effectiveness of immunotherapy to treat cancer, more studies with focus on possible side effects of this method are needed [3]. The results of this study showed that probably immunotherapy might impress virulence factors of microbial flora. In addition, over expression of two major virulence genes in *E.faecalis* of patient's flora which is caused by 6 month immunotherapy were observed that most likely it is due to ability of *E.faecalis* to infect patients. Also this survey has showed ΔCT mean *esp* & *gelE* genes decreased in patients group after immunotherapy. Levels of these mentioned genes increased in group of the patients who returned with pain and inflammation of the prostate. Probably patients' prostate inflammation after immunotherapy is associated with ΔCT mean level of these genes and over expression of these genes may consider as a marker for over function in pattern of pathogenicity.

Major treatments such as chemotherapy, radiotherapy and immunotherapy managed to change regulation of several pathways and mechanisms of body that are not directly objective or related to these treatments. Although normal microflora has serious roles in several vital mechanisms of human but it seems that studies of side effects of treatment methods in microflora were strongly ignored. Correlation of expression of *gel E* and *esp* genes had been approved by the studies antimicrobial resistance that both of the genes had showed over expression [9,12]. Antibiotic therapy of infections could persuade increase express of microflora's pathogenic genes, but side effects of plenty new methods such as immunotherapy aren't clarified yet [13]. There have been some reports about viral and bacterial infections in patients with cancer after recovery by radiotherapy and chemotherapy that mostly lay to cancer care approaches, though might be associated to the microflora alterations too [14-16].

Present study were showed the first evidences of possible effects of adoptive T cell therapy type of immunotherapy in virulence capability of normal microbial flora in patients with prostate cancer. Two advantages may strength the results provided by this study. First, the group of prostate cancer patients and normal subjects had same living conditions, for this purpose, samples of normal subjects selected from related persons of patients who also lived with them because microbial flora is strongly related to life style of individuals. Second advantage is that, instead of the two groups of

Gene	Forward primer	Reverse primer
gelE	5′- GTGACGAAGGTGGTTTCGCT-3′	5′- AAGAGGCAGCATCCATAGCA-3′
Esp	5′- GTGCGCGAAAATCAACACT-3′	5′-CATTTTTACGCATCCGGACTA-3′
16s rRNA	5′- TCAGCAGGGAAGAAGCGAAA-3′	5′- CCTACGAGCTCTTTACGCCC-3′

Table 1: primers of *gelE*, *esp* and *16srRNA*.

Age	PSA	GS	ABCD stage
69 ± 8.5	19.6 ± 8.5	37 patients : 6 GS 16patients : 7 GS 15 patients : 8 GS	38 patients : stage B 30 patients : stage C

PSA: prostate specific antigen, GS: Gleason scale, ABCD stage: "A" and "B" stage means prostate cancer is confined, "C" stage means prostate cancer has grown out of the prostate but has not spread to lymph nodes and "D" stage means cancer has spread to lymph nodes or other tissues.

Table 2: Demographic and clinical data for 68 selected patients.

Gene	Patients before treatment vs. normal	Patients after treatment vs. patients before treatment	Patients after treatment vs. normal
gelE	*p* value: 0.87 *Mean ratio*:1.02	***p* value: 0.02** *Mean ratio*:1.33	***p* value: 0.02** *Mean ratio*:1.37
Esp	*p* value: 0.56 *Mean ratio*:0.97	***p* value: 0.03** *Mean ratio*:1.36	***p* value: 0.03** *Mean ratio*:1.36

Table 3: Gene expression comparisons P-*values* and mean ratio.

patients who were treated by immunotherapy and group that were not treated, were used the strategy before and after treatment to be avoid of wrong results.

Conclusion

While immunotherapy of cancer could cure the ability of cellular immunity also may increase the virulence of normal microflora, so a period of antibiotic therapy at the same or after Immunotherapy of cancer likely succeeds in preventing the feasible infections or constructing of biofilms by microflora. In addition, relinquishing of possible side effects of immunotherapy when this therapy method become the most used method for treatment cancer may be hazardous. Long term studies about side effects of immunotherapy in greater sample size of prostate cancer or other cancer patients will aid to find more elucidated evidence concerning of immunotherapy's disadvantages and advantages, furthermore, comprehensive studies of expression analysis of all of pathogenicity genes of microflora could most likely useful to demonstrate immunotherapy effects on microflora and contingency of future infections in cancer patients after treatment.

Limitations

Lack of study of other virulence genes in *E.faecalis* and low sample size could be the major limitations of study.

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The authors declare that they have no competing interests. This study financially supported by Islamic Azad University.

References

1. Vatsan RS, Bross PF, Liu K, Theoret M, De Claro AR, et al. Regulation of immunotherapeutic products for cancer and FDA's role in product development and clinical evaluation (2013) J Immunother Cancer 1: 5.
2. Gerritsen WR. The evolving role of immunotherapy in prostate cancer (2012) Ann Oncol 23 Suppl 8: 22-27.
3. Hoos A. Evolution of end points for cancer immunotherapy trials (2012) Ann Oncol 23 Suppl 8: 47-52.
4. Seo Y, Lee G. Antimicrobial Resistance Pattern in *Enterococcus faecalis* strains isolated from expressed prostatic secretions of patients with chronic bacterial prostatitis (2013) Korean J Urol 7: 477-481.
5. Comerlato CB, Resende MC, Caierao J, d'Azevedo PA. Presence of virulence factors in *Enterococcus faecalis* and *Enterococcus faecium* susceptible and resistant to vancomycin (2013) Mem Inst Oswaldo Cruz 108: 590-595.
6. Semedo T, Santos MA, Lopes MF, Figueiredo Marques JJ, Barreto Crespo MT, et al. Virulence factors in food, clinical and reference Enterococci: A common trait in the genus? (2003) Syst Appl Microbiol 26: 13-22.
7. Bittencourt de Marques E, Suzart S. Occurrence of virulence-associated genes in clinical *Enterococcus faecalis* strains isolated in Londrina, Brazil (2004) J Med Microbiol 53: 1069-1073.
8. Soares RO, Fedi AC, Reiter KC, Caierao J, d'Azevedo PA. Correlation between biofilm formation and gelE, esp, and agg genes in *Enterococcus spp*. clinical isolates (2014) Virulence 5: 634-637.
9. d'Azevedo PA, Dias CA, Teixeira LM. Genetic diversity and antimicrobial resistance of enterococcal isolates from Southern region of Brazil (2006) Rev Inst Med Trop Sao Paulo 48: 11-16.
10. Lee WS. Improved procedure for identification of group D enterococci with two new media (1972) Appl Microbiol 24: 1-3.
11. Rathnayake I, Hargreaves M, Huygens F. SNP diversity of *Enterococcus faecalis* and *Enterococcus faecium* in a South East Queensland waterway, Australia, and associated antibiotic resistance gene profiles (2011) BMC Microbiol 11: 201.
12. Wang L, Dong M, Zheng J, Song Q, Yin W, et al. Relationship of biofilm formation and gelE gene expression in *Enterococcus faecalis* recovered from root canals in patients requiring endodontic retreatment (2011) J Endod 37: 631-636.
13. Sullivan A, Edlund C, Nord CE. Effect of antimicrobial agents on the ecological balance of human microflora (2001) Lancet Infect Dis 1: 101-114.
14. Nicolatou-Galitis O, Athanassiadou P, Kouloulias V, Sotiropoulou-Lontou A, Dardoufas K, et al. Herpes simplex virus-1 (HSV-1) infection in radiation-induced oral mucositis (2006) Support Care Cancer 14: 753-762.
15. Fijlstra M, Ferdous M, Koning AM, Rings EH, Harmsen HJ, et al. Substantial decreases in the number and diversity of microbiota during chemotherapy-induced gastrointestinal mucositis in a rat model (2015) Support Care Cancer 23: 1513-1522.
16. Himi T, Kukuminato Y, Kita H, Yoshioka I, Kataura A. Effect of radiotherapy on the levels of secretory immunoglobulin A against indigenous and virulent streptococci (1997) Otolaryngol Head Neck Surg 117: 433-437.