Cytopathic effects and local immune responses in repeated neoadjuvant HSV-tk + ganciclovir gene therapy for prostate cancer

Nobuyuki Yanagisawa, Takefumi Satoh, Ken-ichi Tabata, Hideyasu Tsumura, Yasutomo Nasu, Masami Watanabe, Timothy C. Thompson, Isao Okayasu, Yoshiki Murakumo, Shiro Baba, Masatsugu Iwasura

Department of Pathology, St. Marianna University School of Medicine Yokohama-City Seibu Hospital, Yokohama, Kanagawa, Japan
Department of Pathology, Kitasato University School of Medicine, Sagamihara, Kanagawa, Japan
Department of Urology, Kitasato University School of Medicine, Sagamihara, Kanagawa, Japan
Takefumi Satoh Prostate Clinic, Machida, Tokyo, Japan
Department of Urology, Okayama University, Okayama, Japan
Center for Innovative Clinical Medicine, Okayama University Hospital, Okayama, Japan
Department of Genitourinary Medical Oncology - Research, Division of Cancer Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX, USA
Division of Nutrition, School of Health Care, Kiryu University, Midori-City Gunma, Japan

Objective: Cytopathic effects and local immune response were analyzed histologically in prostatic cancer (PCa) with in situ herpes simplex virus-thymidine kinase (HSV-tk)/ganciclovir (GCV) gene therapy (GT).

Methods: Four high-risk PCa patients who received HSV-tk/GCV GT were investigated. After two cycles of intraprostatic injection of HSV-tk and administration of GCV, radical prostatectomy was performed. Formalin-fixed, paraffin-embedded sections were evaluated using immunohistochemistry. PCs with hormone therapy (HT, n = 3) or without neoadjuvant therapy (NT, n = 4) that were equivalent in terms of risk were also examined as reference. Immunoreactively-positive cells were counted in at least three areas in cancer tissue. Labeling indices (LI) were calculated as percentage values.

Available online at www.sciencedirect.com

Asian Journal of Urology (2021) 8, 280–288

Peer review under responsibility of Second Military Medical University.

https://doi.org/10.1016/j.ajur.2020.06.004
2214-3882/© 2021 Editorial Office of Asian Journal of Urology. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
Results: ssDNA LI in GT increased, indicating apoptosis, as well as tumor-infiltrating lymphocytes and CD68-positive macrophages, compared with their biopsies. GT cases showed significantly higher numbers of single-stranded DNA (ssDNA) LI, CD4/CD8-positive T cells and CD68-positive macrophages including M1/M2 macrophages than HT or NT cases. However, there was no significant difference in CD20-positive B cells among the types of case. There were strong correlations between CD8+ T cells and CD68+ macrophages (p = 0.656, p < 0.0001) as well as CD4+ T cells and CD20+ B cells (p = 0.644, p < 0.0001) in PCa with GT.

Conclusions: Enhanced cytopathic effect and local immune response might be indicated in PCa patients with HSV-tk/GCV gene therapy.

© 2021 Editorial Office of Asian Journal of Urology. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Prostatic cancer (PCa) was the most common cancer worldwide in men, accounting for 17% of all cancer cases among men, with 1.6 million cases in 2015 [1]. The incidence of PCa and its mortality rates have increased throughout the world [2]. Currently available therapies—potentially curative localized therapy (radical prostatectomy or irradiation) for PCa are limited [3] or palliative androgen ablation therapy for advanced disease [4]. Despite significant progress in the detection and treatment of PCa, the problem of high relapse rates following radical prostatectomy remains unsolved; for example, a large number of PCa patients undergoing hormone therapy (HT) often show disease progression with castration-resistant status. Because combined radical prostatectomy with adjuvant or neoadjuvant treatment may improve the relapse rate in PCa patients, various adjuvant/neoadjuvant treatments have been examined, and novel therapeutic options are strongly needed.

Suicide gene therapy (GT) is an innovative approach that can kill tumor cells by inserting suicide genes into cancer cells. To date, suicide GT using herpes simplex virus-tyrosine kinase (HSV-tk) gene transduction followed by the systemic administration of ganciclovir (GCV) is one of the novel therapeutic strategies against human malignancies including PCa [5]. GCV phosphorylated by HSV-tk is incorporated into DNA, which inhibits DNA synthesis, leading to cell death [6]. As phase I/II clinical trials, several protocols of GT for PCa have shown biochemical or histological responses [7–13], indicating the effectiveness of GT against PCa. However, because the previous reports of HSV-tk/GCV GT for PCa were usually limited to advanced or refractory cases [10,11], almost no published studies reported histological analysis with HSV-tk/GCV GT, especially in radical prostatectomy specimens. In addition, a single intraprostatic vector injection was performed in previous reports, in contrast with our two cycles of HSV-tk/GCV treatments [7,8].

In the present study, we confirmed the safety of repeated in situ HSV-tk/GCV GT and demonstrated the histological analysis of PCa using radical prostatectomy specimens to shed light on the histological effect of HSV-tk/GCV GT against PCa cells.

2. Patients and methods

2.1. Patient eligibility

Five patients with biopsy-proven, clinically localized PCa were analyzed. All the patients had a Kattan preoperative nomogram score of 115 (high risk of recurrence) [14], and submitted informed consents prior to enrollments in this phase I/II clinical trial. Normal hematopoietic function (platelet count >100 000/mL, neutrophil count >2000/mL and hemoglobin >6.5 mmol/L), a normal coagulation profile, and normal kidney and liver functions (serum creatinine <1.5 mg/dL, bilirubin <2.5 mg/dL, liver enzymes and alkaline phosphatase <2× normal) were required. No androgen deprivation, immunosuppressive drug or corticosteroid were accepted. The protocol used in our study was approved by the Biosafety Committees and the Institutional Review Boards of the participating institutions of the Kitasato University School of Medicine, the Ministry of Health, Labor and Welfare, and Ministry of Education, Culture, Sports, Science and Technology in Japan (Registration number, 07-36V-0001). Informed consents were obtained from all individual participants included in the study.

2.2. Vector and therapy administration

The vector used was a serotype Ad5 adenovirus that contained the HSV-tk gene and Rous sarcoma virus long terminal repeat promoter in the region of the excised E1/E2 wild-type adenoviral genes. This replication-defective adenoviral vector was constructed as described previously [12]. A clinical-grade preparation was made by the Baylor Center for Cell and Gene Therapy, Gene Vector Laboratory (Houston, TX, USA) under good manufacturing practice conditions. On Day 0, a transrectal ultrasound (TRUS) guided single intraprostatic injection of Ad5 HSV-tk vector was delivered in the target lesion mapped by template-guided three-dimensional mapping biopsy [15]. All patients received a total dose of 2×1011 viral particles of the vector. Thereafter patients received an intravenous infusion of 5 mg/kg of GCV (Mitsubishi Tanabe Pharma Corp., Osaka, Japan) twice daily from Day 1 to Day 14 for one course. The intraprostatic vector injection was repeated on
Day 14 and patients received same dose of CGV twice daily from Day 15 to Day 28 for second course. Patients underwent an extended pelvic lymph node dissection and a retro-pubic radical prostatectomy on Day 56 (Fig. 1).

2.3. Monitoring viral DNA detection and neutralizing antibody

Adenoviral DNA in blood was determined by real-time PCR on Days 0, 2, 7, 14, 16, 21, 28, 42 and 56. Gene expression in urine was measured with PCR/real-time PCR as in the above schedule. PCR and real-time PCR were analyzed in a commercial based laboratory (SRL, Tokyo, Japan). Neutralizing antibody titers were determined by neutralization tests in a commercial-based laboratory (SRL) on Days 0, 14, 28, 42 and 56.

2.4. Cytopathic effects and local immune response

All of the resected specimens were fixed in 10% buffered formalin and were step-sliced at 4-mm thickness and processed for embedding in paraffin as a whole mount. Then, 4 μm-thick sections were cut and used for hematoxylin-eosin (H.E.) staining and immunohistochemical analyses. Briefly, tissue sections were deparaffinized and endogenous peroxidase was blocked with 1% hydrogen peroxide in methanol for 30 min. The sections were incubated with primary antibodies, anti-CD20 for B-cells (clone L26, 1/400 diluted, Dako, Carpinteria, CA, USA), CD4 for helper T-cells (1F6, 1/40, Leica Biosystems, Newcastle Upon Tyne, UK), CD8 for cytotoxic T-cells (C8/144B, 1/100, Dako), CD68 for macrophages (PG-M1, 1/200, Dako), anti-CD11c (ab52632, 1/500, Abcam, Cambridge, UK) for M1 macrophages, anti-CD163 (sc-20066, 1/100, Santa Cruz Biotechnology, Dallas, TX, USA) for M2 macrophages and anti-Ki-67 (1/100, Dako) at 4 °C overnight, with or without microwave oven pretreatment to retrieve antigenic reactivity. After incubation with peroxidase-labeled polymer (ChemMate EnVision kit, Dako) for 30 min, 3,3'-diaminobenzidine (DAB) was applied as the chromogen. Nuclei were counterstained with Mayer’s hematoxylin to facilitate histological assessment.

To evaluate apoptotic cells, single-stranded DNA (ssDNA) immunostaining was performed using anti-ssDNA antibody (1/400, IBL Co. Ltd., Gunma, Japan), as described above. Anti-Cytokeratin (CAM5.2, BD Biosciences, San Jose, CA, USA) and anti-prostate-specific antigen (anti-PSA) antibodies (Dako) were also used to evaluate cellular viability with 3-amino-9-ethylcarbazole (AEC, for the chromogen).

2.5. Evaluation of the immunohistochemical staining

Evaluation of immunohistochemical staining was performed in each cancer lesion in the all whole-mount slides. CD20, CD4, CD8, CD68, CD11c and CD163-positive cells per 1 mm² were counted. ssDNA and Ki-67-positive cells were counted in at least 1 000 of epithelial cells, and labeling indices (LIs) were calculated as percentage values. At least five lesions of carcinoma and two of non-cancerous lesions in prostatectomy specimens were analyzed. The biopsy samples of two GT cases (Case 1 and 2, two cores each) were also evaluated. PCa with HT (n=3) or without neo-adjuvant therapy (NT, n=4) that were equivalent to in risk were also examined, as a reference. Affected areas within the tumors were outlined on whole-mount slides and quantified using ImageJ software (U. S. National Institutes of Health, Bethesda, Maryland, USA).

2.6. Statistical analysis

Data were given as the mean±standard deviation values. Comparisons between groups were conducted with the Mann-Whitney U test or the Fisher protected least significant difference test. Relations between each values and ssDNA LI were analyzed using the Spearman’s rank correlation coefficient test. StatView software (version 5.0, Abacus Concepts, Inc. Berkeley, CA, USA) was employed for all statistical analyses, and a p-value less than 0.05 was considered to indicate statistical significance.

2.7. Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committees and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards (approval number: ITI04-1).

3. Results

3.1. Patient characteristics

The patients’ characteristics are shown in Table 1. One patient (Case 3) was switched to HT instead of radical prostatectomy because of prolonged activated partial thromboplastin time. Thus, histological analyses were performed in four patients. After prostatectomy, one patient (Case 2) died as a result of pulmonary thrombus. Fig. 1 Figure 1 Serum PSA levels in prostatic cancer patients treated with HSV-tk/GCV gene therapy. The PSA levels decreased after increasing temporarily at HSV-tk injections. PSA, prostate-specific antigen; GCV, ganciclovir; HSV-tk, herpes-simplex virus-tyrosine kinase.
shows the serum PSA levels in GT cases. The PSA levels decreased after increasing temporarily after HSV-tk injections. The average PSA reduction rate (PSAR) at prostatectomy (56 days) was 23.1% (range, 1.7%–32.0%). The interferon responses were observed in the GT patients, which were described previously [9]. No biochemical recurrence has been observed in the four alive patients (the average follow-up period was 114 months).

3.2. Safety and feasibility

No adenoviral DNA was detected in the blood, in the urine and in the nasal cavity by real-time polymerase chain reaction (PCR) on Day 0, Day 2, Day 7, Day 16, Day 21, Day 28, Day 42 and Day 56. Patients experienced clinical grade 1 toxicity (five patients, six events) and clinical grade 2 toxicity (three patients, three events) with fever within the 24 h following the viral injection. The increase in body temperature never lasted longer than 72 h. Elevation of C-reactive protein values was observed at the four of vector injection in five patients. Temporary elevation of AST was seen as clinical grade 1 toxicity (four patients, five events) and clinical grade 3 toxicity (one patient, one event) following the viral injection, that spontaneously returned to normal level. Temporary elevation of ALT was seen as clinical grade 1 toxicity (two patients, three events) and clinical grade 3 toxicity (one patient, one event) following the viral injection that spontaneously returned to normal level. An asymptomatic grade 1 thrombocytopenia was seen in two patients that spontaneously returned to normal level. No patient developed urinary obstruction. As for symptomatic adverse events, one patient showed grade 2 complications with pollakiuria on Day 4. Grade 1 hematuria was observed in one patient on Day 1. Grade 1 constipation was observed in one patient on Day 3, and grade 1 diarrhea was observed in one patient on Day 16.

3.3. Histology

Generally, moderate to severe chronic inflammation was observed in the cases of prostate treated with GT, predominantly in their peripheral zone. Inflammatory cell infiltration into periprostatic fat was also observed. Neuritis and venulitis were found occasionally. An injection site with small coagulative necrotic focus was identified in one case. In addition, inflammatory cell infiltration was also observed in the tumor. The cancer cells partially lost glandular structure and had pyknotic nuclei (Fig. 2A). Apoptotic bodies were scattered (Fig. 2A, arrows). These findings were thought of as a cytopathic effect. Volumetric analyses with the cytopathic effect were shown as tumor affected area (%) in Table 1 (range, 9.5%–52.7%).

Immunohistochemically, cancer cells with cytopathic effects were still weak and partially positive for cytokeratin (CAM5.2); however, they were mostly negative for PSA (Fig. 2B and 2C). ssDNA-positive cells were also observed (Fig. 2D). Many CD8+ T cells (Fig. 2E) and CD68+ macrophages (Fig. 2F) were observed in tumor tissue, while CD20+ B cells were scant (Fig. 2G). In addition, CD11c+ M1
macrophages were mainly observed rather than CD163+ M2 macrophages (Fig. 2H and I). ssDNA in cancer cells tended to be high compared with that in non-cancerous epithelial cells (Fig. 3). In contrast, the number of inflammatory cells such as CD20+ B cells were significantly increased in non-cancerous lesions compared with in intratumoral lesions (Fig. 3). Other markers including CD4+, CD8+ T cells and CD68+ macrophages also tended to increase more in non-cancerous lesions than in tumor lesions (Fig. 3).

The prostatic biopsy samples (two cores each) of two GT patients (Cases 1 and 2) were also analyzed. The ssDNA LI and intratumoral inflammatory cells in prostatectomy (RP) samples of GT patients increased compared with their biopsies (BP) (ssDNA, 0.1±0.1 vs. 4.9±5.2; CD4, 30.0±13.4 vs. 49.9±39.0; CD8, 36.5±7.9 vs. 246.7±132.4; CD68, 21.5±7.7 vs. 58.6±37.2; CD20, 0.0±0.0 vs. 14.9±38.4, respectively. Fig. 4).

3.4. Comparison among PCAs with or without neoadjuvant therapies

Fig. 5 shows cytopathic effects and local immune responses in PCAs with GT, HT and without NT. Intratumoral CD4+ (GT, 49.9±39.0; HT, 11.1±11.2; NT, 47.5±55.7, respectively. Fig. 5A), CD8+ (GT, 246.7±132.4; HT, 136.4±73.4; NT, 112.7±106.6) T cells and CD68+ macrophages (GT, 58.6±37.2; HT, 14.6±12.2; NT, 6.2±7.0) also increased in GT cases compared with in other groups. However, no significant differences were found in CD20+ B cells among the groups (GT, 14.9±38.4; HT, 14.3±22.4; NT, 17.0±44.7). These results indicate that GT induced apoptosis in the cancer cells and enhanced local immune response with CD8+ T cells and CD68+ macrophages. In addition, both CD11c+ (M1) and CD163+ (M2) macrophages increased significantly in GT cases compared with that in other groups (CD11c, GT, 109.9±32.3; HT, 48.3±9.2; NT, 41.9±18.0. CD163, GT, 4.5±6.2; HT, 0.5±0.8; NT, 2.1±2.6. Fig. 5B). ssDNA LI was higher in GT than the other groups (GT, 4.9±5.2; HT, 0.9±1.0; NT, 1.8±3.0, respectively. Fig. 5B), although Ki-67 LI in GT was not significant (GT, 3.0±2.0; HT, 1.6±1.3; NT, 4.7±2.6).

3.5. Correlation of intratumoral inflammatory cells in PCAs with gene therapy

There were strong correlations between CD8+ T cells and CD68+ macrophages (ρ=0.656, p<0.0001) as well as CD4+ T cells and CD20+ B cells (ρ=0.644, p<0.0001) in PCAs with gene therapy.

Figure 2 Histological results of prostatic cancer patients with HSV-tk gene therapy. (A) The cancer cells showed glandular dissolution, nuclear pyknosis and apoptotic bodies (arrows). Inflammatory cell infiltration was also observed. (B) The cancer cells almost lost PSA positivity. (C) Cytokeratin (CAM5.2) was still focally positive. (D) ssDNA indicating apoptosis was scattered positive in cancer cells. (E–I) Increased CD8+ T cells (E), CD68+ (F) and CD11c+ M1 macrophages (H) were observed in tumor, while CD20+ B cells (G) and CD163+ M2 macrophages (I) were a few. Original magnification, ×400. Scale bars, 50 μm. PSA, prostate-specific antigen; HSV-tk, herpes-simplex virus-tyrosine kinase.
However, no significant positive correlations were found between ssDNA LI and the inflammatory cells. In addition, no significant correlations like in GT were found neither in NT nor HT cases (data not shown).

4. Discussion

In the present study, we proposed a new protocol of HSV-tk/GCV GT for high-risk PCa patients. The characteristics of our protocol were two cycles of HSV-tk/GCV treatments in patients with localized PCa with a high risk of recurrence. We adopted the repeat treatments because a single HSV-tk/GCV cycle was insufficient to induce a histological cytopathic effect, according to previous reports [7,13]. Therefore, we tested two cycles and tried confirming their histologic effects in prostatectomy specimens. Although some GT cases had side effects including mild fever and liver dysfunction, our clinical trial confirmed the safety profile of repeated cycles of HSV-tk/GCV treatment.

Serum PSA reduction at radical prostatectomy (56 days) was observed in all GT cases (Fig. 1). However, some authors previously reported that PCa patients with HSV-tk/GCV GT did not always show PSA reduction. For example, Miles et al. [10] demonstrated that 28 of 36 (77.8%) PCa patients with unsuccessful radiotherapy had a mean 28% PSAR (range, 4.0%–84.8%). Nasu et al. [11] found that six of nine (67%) PCa patients with local recurrence after hormonal therapy had a median 24.1% PSAR (6.7%–43.9%). These PSAR values were almost equal to our result (mean 23.1%), in spite of repeated HSV-tk/GCV treatment against non-refractory localized PCa. Unexpectedly, our new protocol could not confirm further effects, at least in the PSAR data. In the present study, we first demonstrated histological analyses using the radical prostatectomy specimens.
in PCa with repeated HSV-tk/GCV GT; however, further study is needed because no histological comparison between single and repeated HSV-tk injection has been reported.

We found a cytopathic effect using H.E. slides and ssDNA LI in radical prostatectomy specimens with HSV-tk/GCV GT, in line with the previous reports [7,8]. However, the authors of one study reported that no viral cytopathic effect was observed in four PCa patients with GT [13]. Our PSA data and histological result including affected tumor area also indicated individual differences among the patients, suggesting that HSV-tk/GCV GT is not always effective in PCa patients. We focused on Case 4, in whom strong cytopathic effect, large affected tumor area (52.7%) and increased ssDNA LI were observed compared with other GT cases. However, a significantly increased PSAR was not observed (27.7%) compared with other GT cases. Although the reason for this is unclear, it may be possible that Cases 3 and 4 had lower anti-adenovirus neutralizing antibody responses (16 and 32, respectively, Table 1), suggesting that the injected vector may still retain effectiveness against PCa.

We hypothesized that GT caused both cytopathic effects including apoptosis and local immune responses. However, we could not find a significant close correlation between ssDNA LI (apoptosis) and local immune response. To our knowledge, our study is the first to describe this result. Although the reason is still unclear, the increasing apoptosis induced by HSV-tk vector may be a different phenomenon from the local immune response caused mainly by intraprostatic viral injection. Our results showed that ssDNA LIs were higher in the carcinoma cells than in the non-cancerous cells [Fig. 3], in the radical prostatectomy specimens than the biopsy [Fig. 4] and in the GT cases than NT and HT [Fig. 5B]. Taking into consideration that HSV-tk/GCV GT induces suicide in vector-transfected cells, we confirmed the “direct” cytopathic effect in carcinoma cells in the GT cases. Local immune responses may indicate the “indirect” effects caused by HSV-tk/GCV GT.

A close correlation between CD8+ T cells and CD68+ macrophages in PCa with GT was observed (Table 2). The previous report also pointed out that CD8+ and CD68+ cells increased in PCa with GT compared with controls [7]. In other tumors, CD8+ cells that are functionally characterized as cytotoxic T lymphocytes have been most often associated with favorable prognosis [16,17]. However, intratumoral CD68+ cells including tumor-associated macrophages (TAM) have been reported as both promoting and preventing tumor progression [10]. CD8+ cytotoxic T cells kill their targets by programming them to induce apoptosis [19]. Classically activated macrophages are known to have a high bactericidal and tumoricidal capacity [18]. Considering these facts, it seems reasonable that the positive correlation between CD8+ and CD68+ cells indicates the

**Table 2 Correlation coefficients for ssDNA and intratumoral inflammatory cells in prostatic cancer patients with HSV-tk/GCV gene therapy.**

| marker     | \( \rho^a \) | \( p\)-Value\textsuperscript{b} |
|------------|---------------|-------------------------------|
| ssDNA/CD4  | -0.322        | 0.0271                        |
| ssDNA/CD8  | -0.084        | 0.5642                        |
| ssDNA/CD68 | -0.193        | 0.1853                        |
| ssDNA/CD20 | -0.474        | 0.0012                        |
| CD4/CD8    | 0.466         | 0.0014                        |
| CD4/CD68   | 0.483         | 0.0009                        |
| CD4/CD20   | 0.644         | <0.0001                       |
| CD8/CD68   | 0.656         | <0.0001                       |
| CD8/CD20   | 0.381         | 0.0091                        |
| CD68/CD20  | 0.392         | 0.0072                        |

\( \text{ssDNA, single strand DNA; HSV-tk/GCV, herpes-simplex virus-tyrosine kinase/ganciclovir.} \)

\textsuperscript{a} Statistical analysis was done using Spearman’s rank correlation coefficient test.

\textsuperscript{b} \( p\)-Value <0.05 was considered significant.
elimination of tumor cells. Furthermore, a correlation between CD20+ and CD4+ cells was also observed. B lymphocytes are associated with a humoral immune reaction including antibody production. The production of neutralizing antibodies by plasma cells and memory B cells is dependent on sequential CD4+ T cell to drive antibody affinity maturation and memory formation [20]. This may be one of the reasons for the close correlation between CD20+ and CD4+ cells. However, GT Case 5 with high antiadenovirus antibody titer (1024) did not significantly increase CD20+ B cells compared with others. Further study is needed.

PCa with GT showed increased ssDNA LI as well as CD8+ T cells and CD68+ macrophages (predominantly CD11c+ M1 macrophages) compared with NT or HT patients with significance (Fig. 5), indicating that GT treatment strongly induced cytopathic effect and local immune response in the PCa. Interestingly, a previous report also demonstrated induced cytopathic effect and local immune response in the macrophages (predominantly CD11c+ M1 macrophages) compared with controls [21]. Although these therapeutic mechanisms are thought to be different, it is interesting that both GT and HT showed partly similar tendencies with respect to local immune responses. As a local immune response, we investigated tumor-infiltrating lymphocytes (TIL) and M1/M2 macrophages in PCa. TIL has been believed to be one of the host-mediated anticancer responses in the microenvironment and has been reported to be a prognostic factor in malignancies. In addition, the presence of TIL also influenced the neo-adjvant chemotherapeutic response in breast cancers [22,23]. Similarly, GT using intraprostatic viral injection was thought to induce the forced recruitment of CD8+ lymphocytes and CD68+ macrophages into PCa. We confirmed that the local immune responses and cytopathic effect were increased in the prostatectomy specimens after GT compared with the biopsy specimens (Fig. 4). Thus, GT may enhance cytotoxic response, resulting in anti-tumor immunity.

We found that ssDNA LI was high in cancer cells compared with non-cancerous prostatic epithelial cells in GT despite their inflammatory cell infiltration (Fig. 3). GCV inhibits DNA synthesis mainly in cells having high proliferative activities such as cancer cells [24], supporting their enhanced apoptosis. In addition, this GT effect may be more selectively caused by the adenovirus vector localization; no vector was detected in all of the GT patients' peripheral blood, urine and nasal mucosae. This might be an advantage of GT in terms of selective cytopathic effect against PCa.

Previous reports indicated a “bystander effect” of HSV-tk/GCV treatment [25]. This was an additional cytopathic effect that occurred in tumor cells expressing the HSV gene as well as their adjacent cells without HSV-tk induction. Because of the high cellularity of tumor cells, this phenomenon may cause increased cytopathic effect, especially in high-grade malignancies. In addition, we also demonstrated a clear increase of central memory CD8+ T cells and tumor antigen-specific T cells in peripheral blood following HSV-tk/GCV treatment [9]. This was expected given the systemic immune response effects against the PCa as well as the metastatic cancer foci. Therefore, HSV-tk/GCV GT has potential as a new treatment, especially against unresectable malignancies such as advanced cancer with metastasis or high-grade brain tumors. Indeed, some clinical GT trials using HSV-tk/GCV have been reported against high-grade gliomas [26].

HSV-tk/GCV GT may also have some disadvantages. First, some authors found differing effectiveness among patients, as was the case in this study. PSA recurrence occurred in seven of 18 (39%) patients, and no PSA reduction was observed in three of eight (38%) PCa patients treated with GT [10,11]. Although all of our GT cases showed PSA reduction, a wide range of PSARs from 1.7% to 32.0% were observed, consistent with previous reports [10,11]. Furthermore, Case 4 showed a strong cytopathic effect, large affected tumor area and high ssDNA LI compared with the other cases (Fig. 3, Table 1), indicating individual variation in their responses to GT. Second, the expected duration of the vector’s action in vivo remains unclear. According to the PSA reduction data, it seems to be about 30–50 days after a single HSV-tk injection [11]. To date, the safety of more than two cycles of HSV-tk/GCV treatment has not been confirmed. Therefore, it is a problem that GT still requires stronger and longer-range medication.

The limitations of this study include its small sample size, the setting of the control cases that did not allow adequate assessment of their effects.

5. Conclusion

In summary, we proposed a new protocol of repeated HSV-tk/GCV gene therapy in patients with localized PCa. Our results demonstrated cytopathic effects and local immune response consistent with some previous reports, indicating that the HSV-tk/GCV GT could be a novel gene therapy against PCa, especially in high-risk patients.

Author contributions

Study concept and design: Nobuyuki Yanagisawa, Takefumi Satoh.
Data acquisition: Nobuyuki Yanagisawa, Takefumi Satoh.
Data analysis: Nobuyuki Yanagisawa.
Drafting of manuscript: Nobuyuki Yanagisawa, Takefumi Satoh.
Critical revision of the manuscript: Isao Okayasu, Yoshiki Murakumo, Shiro Baba, Masatsugu Iwamura.
Performing the clinical treatments: Takefumi Satoh.
Providing the clinical information: Ken-ichi Tabata, Hideyasu Tsumura.
Developing the vector for HSV-tk Gene therapy: Yasutomo Nasu, Masami Watanabe, Timothy C. Thompson.

Conflicts of interest

The authors have no conflict of interest.

Acknowledgements

This work was supported by Grants-in-Aid for Scientific Research (JSPS KAKENHI) grant (number 21592060). We
thank Dr. M. Ichinoe, E. Satoh and Y. Numata for expert technical assistance.

References

[1] Fitzmaurice C, Allen C, Barber RM, Barregard L, Bhutta ZA, Brenner H, et al. Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 32 cancer groups, 1990 to 2015: a systematic analysis for the global burden of disease study. JAMA Oncol 2017;3:524–48.

[2] Center MM, Jemal A, Lortet-Tieulent J, Ward E, Ferlay J, Brawley O, et al. International variation in prostate cancer incidence and mortality rates. Eur Urol 2012;61:1079–92.

[3] Keyes M, Crook J, Morton G, Vigneault E, Usmani N, Morris WJ. Treatment options for localized prostate cancer. Can Fam Physician 2013;59:1269–74.

[4] Westdorp H, Benoist GE, Schers HJ, van Erp PH, Gerritsen WR, Mulders PF, et al. Hormone therapy in prostate cancer; a pharmacotherapeutic challenge. Ned Tijdschr Geneeskd 2015;159:A9250 [Article in Dutch], https://pubmed.ncbi.nlm.nih.gov/26246066/.

[5] Hassan W, Sanford MA, Woo SL, Chen SH, Hall SJ. Prospects for herpes-simplex-virus thymidine-kinase and cytotoxic gene transduction as immunomodulatory gene therapy for prostate cancer. World J Urol 2000;18:130–5.

[6] Moolten FL, Wells JM. Curability of tumors bearing herpes thymidine kinase genes transferred by retroviral vectors. J Natl Cancer Inst 1990;82:297–300.

[7] Ayala G, Satoh T, Li R, Shaley M, Gдорож Y, Aguilar-Cordova E, et al. Biological response determinants in HSV-tk + ganciclovir gene therapy for prostate cancer. Mol Ther 2006;13:716–28.

[8] Ayala G, Wheeler TM, Shaley M, Thompson TC, Miles B, Aguilar-Cordova E, et al. Cytopathic effect of in situ gene therapy in prostate cancer. Hum Pathol 2000;31:866–70.

[9] Kubo M, Satoh T, Tabata KI, Tsumura H, Iwamura M, Baba S, et al. Enhanced central memory cluster of differentiation 8+ and tumor antigen-specific T cells in prostate cancer patients receiving repeated in situ adenovirus-mediated suicide gene therapy. Mol Clin Oncol 2015;3:515–21.

[10] Miles BJ, Shaley M, Aguilar-Cordova E, Timme TL, Lee HM, Yang G, et al. Prostate-specific antigen response and systemic T cell activation after in situ gene therapy in prostate cancer patients failing radiotherapy. Hum Gene Ther 2001;12:1955–67.

[11] Nasu Y, Saika T, Ebara S, Kusaka N, Kaku H, Abarzuza F, et al. Suicide gene therapy with adenoviral delivery of HSV-tk gene for patients with local recurrence of prostate cancer after hormonal therapy. Mol Ther 2007;15:834–40.

[12] Satoh T, Teh BS, Timme TL, Mai WY, Gдорож Y, Kusaka N, et al. Enhanced systemic T-cell activation after in situ gene therapy with radiotherapy in prostate cancer patients. Int J Radiat Oncol Biol Phys 2004;59:562–71.

[13] van der Linden RR, Haagmans BL, Mongiat-Artus P, van Doornum GJ, Kraaij R, Kadmon D, et al. Virus specific immune responses after human neoadjuvant adenovirus-mediated suicide gene therapy for prostate cancer. Eur Urol 2005;48:153–61.

[14] Kattan MW, Eastham JA, Stapleton AM, Wheeler TM, Scardino PT. A preoperative nomogram for disease recurrence following radical prostatectomy for prostate cancer. J Natl Cancer Inst 1998;90:766–71.

[15] Satoh T, Matsumoto K, Fujita T, Tabata K, Okusa H, Tsuboi T, et al. Cancer core distribution in patients diagnosed by extended transperineal prostate biopsy. Urology 2005;66:114–8.

[16] Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Miecnik B, Lagorce-Pages C, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science 2006;313:1960–4.

[17] Mahmoud SM, Paish EC, Powe DG, Macmillan RD, Grainge MJ, Lee AH, et al. Tumor-infiltrating CD8+ lymphocytes predict clinical outcome in breast cancer. J Clin Oncol 2011;29:1949–55.

[18] Sica A, Schioppa T, Mantovani A, Allavena P. Tumour-associated macrophages are a distinct M2 polarised population promoting tumour progression: potential targets of anti-cancer therapy. Eur J Canc 2006;42:717–27.

[19] Chavez-Galan L, Arenas-Del Angel MC, Zenteno E, Chavez R, Lascurain R. Cell death mechanisms induced by cytotoxic lymphocytes. Cell Mol Immunol 2009;6:15–25.

[20] Kerfoot SM, Yaari G, Patel JR, Johnson KL, Gonzalez DG, Kleinstein SH, et al. Germlinal center B cell and T follicular helper cell development initiates in the interfollicular zone. Immunity 2011;34:947–60.

[21] Gannon PO, Poisson AO, Delvoye N, Lapointe R, Mess-Mason AM, Saad F. Characterization of the intra-prostatic immune cell infiltration in androgen-deprived prostate cancer patients. J Immunol Methods 2009;348:9–17.

[22] Denkert C, Loibl S, Noske A, Roller M, Muller BM, Komor M, et al. Tumor-associated lymphocytes as an independent predictor of response to neoadjuvant chemotherapy in breast cancer. J Clin Oncol 2010;28:105–13.

[23] Yamaguchi R, Tanaka M, Yano A, Tse GM, Yamaguchi M, Koura K, et al. Tumor-infiltrating lymphocytes are important pathologic predictors for neoadjuvant chemotherapy in patients with breast cancer. Hum Pathol 2012;43:1688–94.

[24] Ezzeddeen ZD, Martuza RL, Platika D, Short MP, Malick A, Choi B, et al. Selective killing of glioma cells in culture and in vivo by retrovirus transfer of the herpes simplex virus thymidine kinase gene. N Biol 1991;3:605–14.

[25] Freeman SM, Abboud CN, Whartenby KA, Packman CH, Koepelin DS, Moolten FL, et al. The ‘bystander effect’: tumor regression when a fraction of the tumor mass is genetically modified. Canc Res 1993;53:5274–83.

[26] Immnenen A, Vapalahti M, Tyylena K, Hurksainen H, Sandmair A, Vanninen R, et al. AdvHSV-tk gene therapy with intravenous ganciclovir improves survival in human malignant glioma: a randomised, controlled study. Mol Ther 2004;10:967–72.