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ORIGINAL PAPER

Trichomonas vaginalis: a possible foe to prostate cancer

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Abstract Prostate cancer (PCA) is the most common malignancy in men in USA, and the role of *Trichomonas vaginalis (T. vag)* in the development of PCA is still controversial. Clonogenic assay, PCNA staining, TUNEL staining and caspase-3 activity assay were used to investigate the in vitro role of *T. vag* in human prostate cancer. We further investigated the possible molecular mechanisms using RT-PCR and immunohistochemical staining. Culture supernatant of *T. vag* inhibits growth of PC-3 prostate cancer cells, and this correlated with upregulation of p21. Culture supernatant of *T. vag* induced apoptosis of PC-3 cells, and this correlated with downregulation of Bcl-2. The growth inhibition effect of culture supernatant of *T. vag* is also demonstrated in another prostate cancer cell line DU145, suggesting that its effect is not specific to one prostate cancer cell line. Culture supernatant of *T. vag* inhibits growth of prostate cancer by inhibition of proliferation and promotion of apoptosis. Such a study might be helpful to address the association between PCA and infection of *T. vag*.

Keywords *Trichomonas vaginalis* · Apoptosis · Proliferation · Prostate cancer

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Introduction

The incidence of prostate cancer (PCA) differs worldwide with the highest rate in USA and the lowest rate in China. PCA is the most common malignancy in men in USA with an estimated 220,800 new cases expected in 2015 [1]. It accounts for 33 % of all newly diagnosed malignant tumors in men in USA and contributes significantly to disease burden due to cancer in USA [1, 2]. However, the etiology and pathogenesis of PCA are still poorly understood [2].

Trichomonas vaginalis (*T. vag*) is an extracellular flagellated parasitic protozoan [3–6]. It is the most common nonviral sexually transmitted pathogen worldwide, with an annual incidence of over three million cases [3–6]. It is estimated that about 50 % of infected women will be symptomatic [3–6]. Although men are generally asymptomatic and are thus regarded as carrier [3–6], it is known that it can cause urethritis and prostatitis in some infected men [7].

T. vag was first suspected to be associated with the development of PCA in 1971 [7], and for decades, a couple of clinical studies seem to support this suspect that *T. vag* is associated with the development of PCA [8–11]. A recent study also indicated that *T. vag* homolog of macrophage migration inhibitory factor induced growth in both benign prostatic hyperplasia epithelial cell line and PC-3 PCA cell line [12]. However, increasing evidence seems to start to challenge this and suggests that *T. vag* might not be associated with the development of PCA [13–15]. Interestingly, some studies even further suggest that *T. vag* might have the ability to directly or indirectly damage normal urogenital epithelial cells including normal prostate epithelial cells [16–20] and induce apoptosis of lung cancer or cervical cancer [21, 22]. Obviously, the association between

T. vag and PCA is not fully understood and further studies are needed to clarify the relationship between *T. vag* and PCA. This study is designed and performed to investigate the direct effect of *T. vag* on two classic PCA cell lines PC-3 and DU145 by using the culture supernatant of *T. vag*.

Materials and methods

T. vag cell culture

T. vag G3 (ATCC PRA-98) was obtained from the American Type Culture Collection. Stock cultures were grown in a trypticase-yeast-extract-maltose (TYM) medium, adapted from that of Hollander and Beal, as described by Nielsen et al. [23–25]. Media were sterilized by autoclave and then frozen at −20 °C. Prior to use, media were completed by the addition of horse serum (Lonza, Allendale, NJ; 10 % v/v final concentration), which had been heat inactivated at 56 °C for 30 min, penicillin–streptomycin solution (50 U/ml penicillin G and 50 μg/ml of streptomycin sulfate), ferrous ammonium sulfate (0.01 mg/ml final concentration) and vitamin B12 (cyanocobalamin, 8 μg/ml final concentration). Routine culture was carried out at 35 °C in 25 cm^2 tissue culture flasks containing 5–10 ml of complete TYM medium. Cell growth and viability were determined by counting the number of intact and motile trichomonads in an aliquot using a Neubauer hemocytometer. All stock cultures were inoculated at a density of 1.0×10^5 cells/ml and were passaged every 48–72 h.

Collection of *T. vag*-conditioned growth medium

Following 48 h of growth, cultures reached an average density of 3.5 x 106 cells/ml. To collect conditioned media, cultures were transferred to 15-mL polypropylene centrifuge tube and cells pelleted using a tabletop centrifuge (1500×*g* for 7 min at 4 °C). Post-centrifugation, conditioned medium, free of cell debris, was collected, immediately frozen using liquid nitrogen and stored at −80 °C. Control media, which had not been inoculated with *T. vag*, were processed in an identical manner.

Tumor cell lines

PC-3 and DU145 prostate cancer cell lines were provided by Dr. Susan L. Deutscher's group and Dr. Dannis B. Lubahn's group (University of Missouri, Columbia, MO). Cancer cells were cultured in DMEM (Invitrogen, Carlsbad, CA, USA), supplemented with 10 % FBS (heat inactivated) and 1 % penicillin–streptomycin (Invitrogen, Carlsbad, CA, USA). Tumor cells were incubated in a humidified 5 % CO_2 incubator (Fisher Scientific, Pittsburgh, PA, USA) at 37 °C. Cells were grown until they reached 70 % confluence, at which time they were ready to experimental treatment.

Treatment of prostate cancer cell lines with *T. vag*-conditioned growth medium

Human PC-3 and DU145 cells (70 % confluent) were treated for 3 days with *T. vag*-conditioned growth medium or medium alone. The dilution ratio for *T. vag*-conditioned growth medium used in this study is based on our pilot experiments. The dilution of 1:10–1:20 was found to be the optimal dilution for a 3-day treatment. Thus, the dilution of 1:15 for *T. vag*-conditioned growth medium was used in this study.

Clonogenic survival assay

Three days after *T. vag*-conditioned growth medium treatment, cells were detached and counted. Clonogenic survival assay was performed as described previously [26–29]. The number of colonies was counted and expressed as a percentage of total colonies in controls.

Immunohistochemistry (IHC)

IHC staining for important proteins such as PCNA, p21, Bcl-2 was described previously [30, 31]. To quantify the number of PCNA + cells, cancer cells from a couple of randomly selected fields (high power) were counted with the assistance by specific image analysis software MetaMorph (Molecular Devices Analytical Technologies, Sunnyvale, CA, USA). Average staining intensity for proteins was measured, and the results are expressed as the average integrated intensity of 3 slides ± SEM relative to that in control cells.

RT-PCR

RNA was extracted after homogenized in TRIzol (Invitrogen). RNA concentration was then determined by nanodrop. In this study, 1 μg RNA was reverse-transcribed as previously described [30, 31]. GAPDH was used in this study to verify whether the equal amount of RNA was amplified. Primer sequences have been described previously [30, 31].

TUNEL staining

Apoptosis was determined by TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling) assay using an Apoptag kit (Chemicon, El Segundo, CA, USA) as previously described [26–29]. To

quantify the number of apoptotic cells, cancer cells in a couple of randomly selected fields (magnification ×400) were counted with the assistance of MetaMorph. TUNEL + cells were expressed as a percentage of total cells.

Measurement of caspase-3 activity

Cellular caspase-3 activity of DU145 and PC-3 cells targeting the sequence DEVD (Asp-Glu-Val-Asp) was measured using a caspase-3/CPP32 colorimetric assay kit (BioVision) as described before [26–29].

Statistics

All experiments were repeated two times. Statistical analysis of data was performed using an unpaired two-tailed Student's *t* test. A p value < 0.05 was considered significant.

Results

Culture supernatant of *T. vag* inhibits growth of human prostate cancer cells

PC-3 prostate cancer cells were used to investigate the direct effect of *T. vag* on prostate cancer. Seventy percentage confluent cells were treated with *T. vag*-conditioned growth medium or medium alone for 3 days, and cell survival was evaluated by clonogenic survival assay. The percentage of colonies of PC-3 was significantly lower after *T. vag* treatment compared with controls treated with medium alone (Fig. 1a, $p < 0.05$). The anti-proliferative effect of *T. vag* on PC-3 cells was also supported with staining for a marker for cell proliferation PCNA and its mRNA (Fig. 1b–d). To exclude the possibility that this effect is PC-3 cell line-specific, the effect of *T. vag* on another typical prostate cell line DU145 was also investigated and similar results were obtained (Fig. 1e–h). These results strongly indicate that *T. vag* inhibits growth and survival of prostate cancer.

Culture supernatant of *T. vag* increased expression of anti-proliferative molecule p21 in PC-3 cells

Cell proliferation is well regulated, and the delicate balance between pro- and anti-proliferative molecules orchestrates this process [32–35]. On one hand, cyclin B, cyclin D, cyclin E, cdk2 and cdk4 are traditionally regarded as major pro-proliferative molecules. One the other hand, p18, p21, p27 and p53 are traditionally regarded as major anti-proliferative molecules [32–35]. To study the potential molecular mechanisms where *T. vag* inhibits growth of PC-3 prostate cancer cells, mRNA expression of major pro-proliferative molecules and anti-proliferative molecules mentioned above was determined first by RT-PCR (Fig. 2). mRNA expression of anti-proliferative molecules was all comparable in both groups ($p > 0.05$) except that of p21 in PC-3 cells whereby its mRNA expression level was significantly higher in *T. vag* group compared to the control group (Fig. 2, $p < 0.05$). This suggests that p21 in PC-3 cells is upregulated by *T. vag*-conditioned growth medium. To further confirm this finding at protein level, IHC staining for p21 was performed and the result echoed the finding by RT-PCR (Fig. 3). These results suggest that upregulation of p21, one of the most anti-proliferative molecule, correlates with the inhibitory effect of *T. vag* on growth and survival of PC-3 prostate cancer cells. mRNA expression of pro-proliferative molecules was all comparable in both groups ($p > 0.05$) except that of cyclin E in PC-3 cells. mRNA expression level of cyclin E was unexpectedly higher in *T. vag* group compared to the control group (Fig. 2, $p < 0.05$).

Culture supernatant of *T. vag* promotes apoptosis of PC-3 cells

Cell growth is the net result of cellular proliferation and cellular apoptosis [32–40]. The inhibitory effect of *T. vag* on growth of PC-3 cells might also attribute to increased apoptosis of PC-3 prostate cancer cells. To address this, PC-3 cancer cells were treated with supernatant of *T. vag* or medium alone for 3 days and TUNEL staining method was used to evaluate cellular apoptosis of PC-3 cancer cells (Fig. 4). A higher number of TUNEL + cells were found after *T. vag* treatment, and the difference in TUNEL + cells was significant compared to the control group (Fig. 4, $p < 0.05$). Next, we used a caspase-3 activity kit to measure the relative caspase-3 activity in PC-3 cells. As expected, the relative caspase-3 activity is significantly higher in *T. vag* group when compared with control group (Fig. 4). These results indicate that *T. vag* promotes apoptosis of PC-3 cells and this might be another contributing factor for the inhibitory effect of *T. vag* on growth of PC-3 cells.

Culture supernatant of *T. vag* decreased expression of anti-apoptotic molecule Bcl-2 in PC-3 cells

Cellular apoptosis is controlled by the fine balance between pro- and anti-apoptotic molecules [36–40]. On one hand, Fas, FasL, TRAIL-R1, TRAIL and Bax are traditionally regarded as major pro-apoptotic molecules. On the other hand, FLIP, Bcl-2 and survivin are the major anti-apoptotic molecules. To investigate the possible molecular mechanisms whereby *T. vag* promotes apoptosis of PC-3 prostate

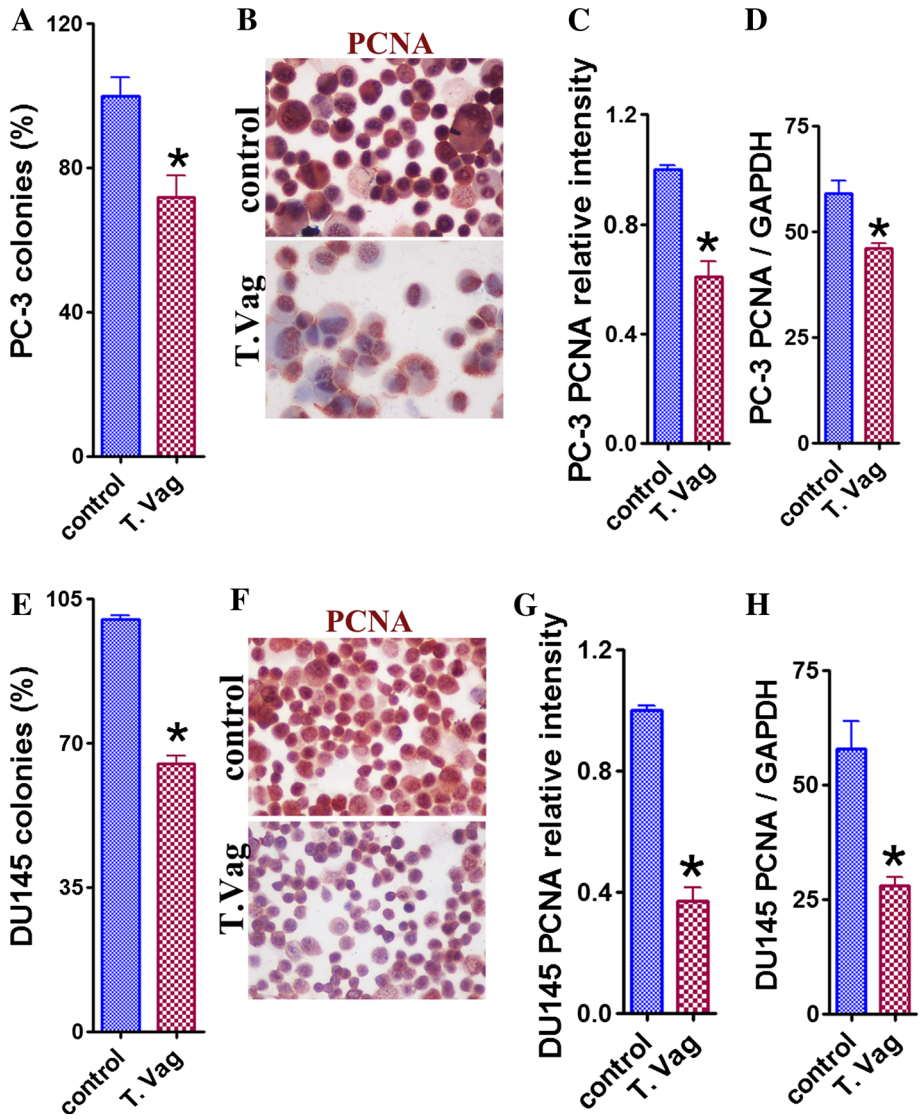

Fig. 1 Culture supernatant of *T. vag* inhibits proliferation of prostate cancer cells. **a** Clonogenic survival assay of PC-3 cells treated with supernatant of *T. vag* (1:15 dilution) or medium alone (control). The number of colonies was counted and expressed as a percentage of total colonies in controls (medium alone). **b** and **c** Representative IHC results for PCNA of PC-3 cells treated with supernatant of *T. vag* or medium alone. PCNA + cells (*red*) in 5–6 randomly selected high-power fields of three slides were counted using MetaMorph software and summarized. **d** RT-PCR evaluation of PCNA mRNA expression in cells treated with supernatant of *T. vag* or medium alone. **e**–**h** Clonogenic survival assay, representative IHC results for PCNA and RT-PCR evaluation of PCNA mRNA expression in cells treated with supernatant of *T. vag* or medium alone in DU145 cells. Results are expressed as the mean OD + SEM in each group and are representative of two independent experiments. A significant difference in the percentage of colonies, PCNA + cells or mRNA expression level, is indicated by the *asterisk* (*T. vag* group vs. control group, $p < 0.05$). Original magnification in **b** and **f**: ×400

cancer cells, mRNA expression of pro- and anti-apoptotic molecules in PC-3 cells was determined by RT-PCR. mRNA expression of these molecules was all comparable in both groups ($p > 0.05$) except that of Bcl-2, one of the critical anti-apoptotic molecules. mRNA expression level of Bcl-2 was significantly lower in *T. vag* group compared to the control group (Fig. 5, $p < 0.05$). To further confirm this finding at protein level, IHC staining for Bcl-2 was performed and the result echoed the finding by RT-PCR (Fig. 6). These results strongly indicate that downregulation of anti-apoptotic molecule Bcl-2 correlates with the increased apoptosis in PC-3 cells induced by *T. vag*.

Discussion

In this study, we asked whether culture supernatant of *T. vag* has any effect on growth of human prostate cancer cells. We found that culture supernatant of *T. vag* inhibits

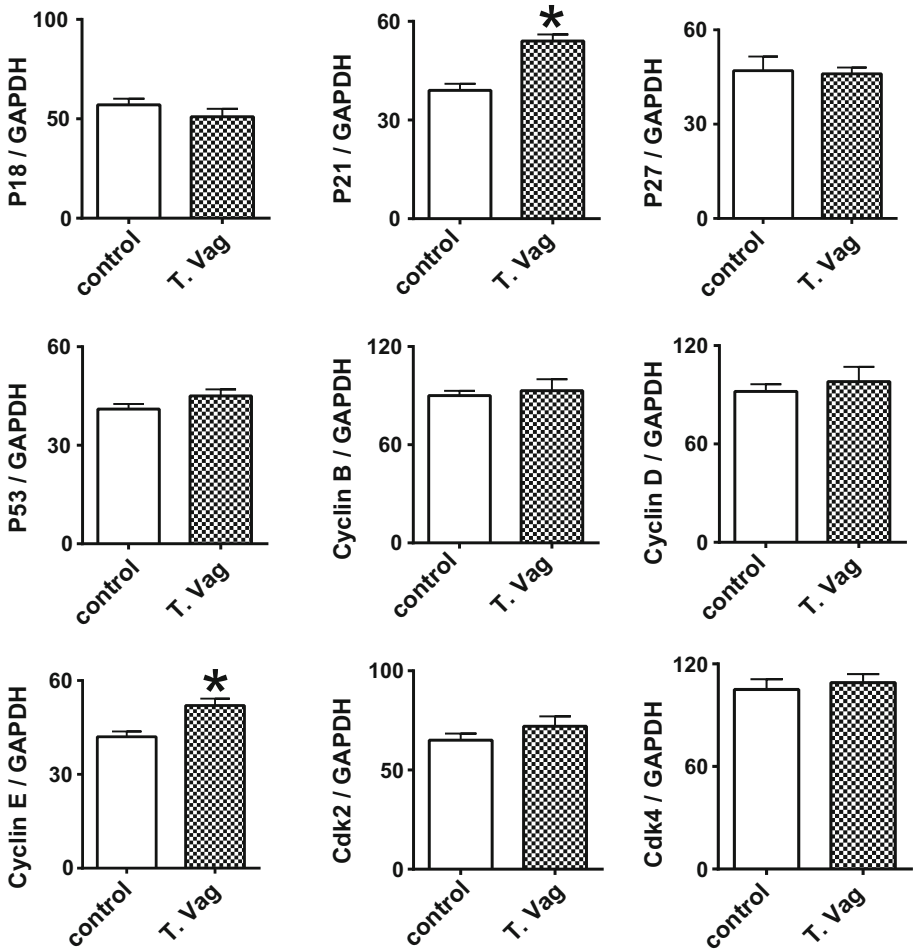

Fig. 2 Effect of culture supernatant of *T. vag* on expression of pro- and anti-proliferative molecules in PC-3 cells evaluated by RT-PCR mRNA was extracted as described in the methods. Experiments are done in triplicate, and results are expressed as the mean ratio of pro- and anti-proliferative molecule densitometric units/GAPDH + SEM (×100) and are representative of two to three independent experiments. A significant difference in mRNA expression between cells treated with culture supernatant of *T. vag* and those in controls is indicated by the *asterisk* ($p < 0.05$)

growth of PC-3 prostate cancer cells and this correlated with upregulation of p21. We further found that culture supernatant of *T. vag* induced apoptosis of PC-3 cells and this correlated with downregulation of Bcl-2. The growth inhibition effect of culture supernatant of *T. vag* is also demonstrated in another prostate cancer cell line DU145, suggesting that its effect is not specific to one prostate cancer cell line. To the best of our knowledge, our study is the first to directly demonstrate that there is a negative association between PCA and infection of *T. vag*.

T. vag, a common extracellular protozoan transmitted sexually, has been suspected to be associated with the development of PCA in 1971 by Dr. Wynder [7]. The reasons for this suspect are the following two facts: (1) its ability to induce prostatitis and (2) its common occurrence. This suspect was further supported by Dr. Gardner's study which showed that atypical hyperplasia of prostate epithelial cells was observed in area near *T. vag* [10, 11]. In the following years, a couple of clinical studies seem to support this suspect that *T. vag* is associated with the development of PCA [8–11]. More interestingly, a recent study is also in line with this by showing that *T. vag* homolog of macrophage migration inhibitory factor induced growth in both benign prostatic hyperplasia epithelial cell line and PC-3 PCA cell line [12]. However, our study is not in consistent with this suspect that there is a positive association between T. vag and PCA. Our study is consistent with the increasing evidence from clinical studies suggesting that *T. vag* is not associated with the development of PCA [13–15]. Our study is also consistent with some studies suggesting that *T. vag* might induce apoptosis of lung cancer or cervical cancer [21, 22]. More interestingly, our study might even suggest that *T. vag* infection might be potentially beneficial to patients in an early neoplastic state.

Cell proliferation is a series of tightly regulated events, and pro- and anti-proliferative molecules are critical in cell proliferation [32–35]. On one hand, cyclin B, D, E and cyclin-dependent kinase 2 and 4 (cdk2 and cdk4) promote cell proliferation, and thus, they are regarded as pro-proliferative molecules. On the other hand, p18, p21, p27 and p53 inhibit cell proliferation, and accordingly, they are regarded as anti-proliferative molecules [32–35]. In this study, we found that the anti-proliferative effect of cultured

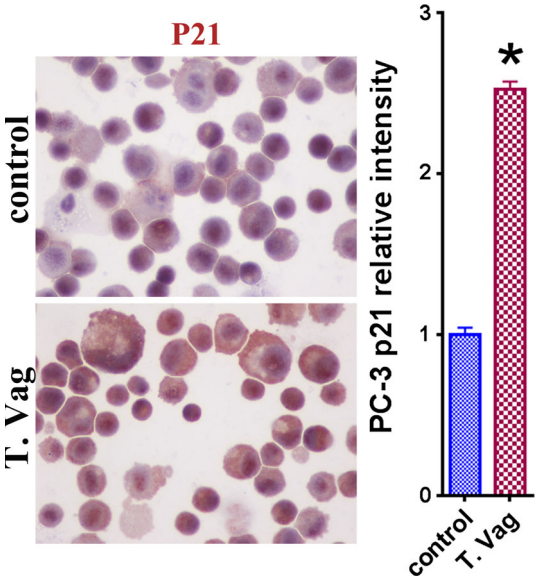

Fig. 3 Effect of culture supernatant of *T. vag* on expression of p21 in PC-3 cells evaluated by IHC. Shown are representative pictures of IHC. The relative staining intensity in 3–5 randomly selected high-power fields of three slides from each group was analyzed by MetaMorph software. Results are expressed as the average integrated staining intensity of three slides + SEM relative to that in control cells. A significant difference in staining intensity between cells treated with culture supernatant of *T. vag* and those in controls is indicated by the *asterisk* ($p < 0.05$). Shown is representative of two independent experiments. Original magnification ×400

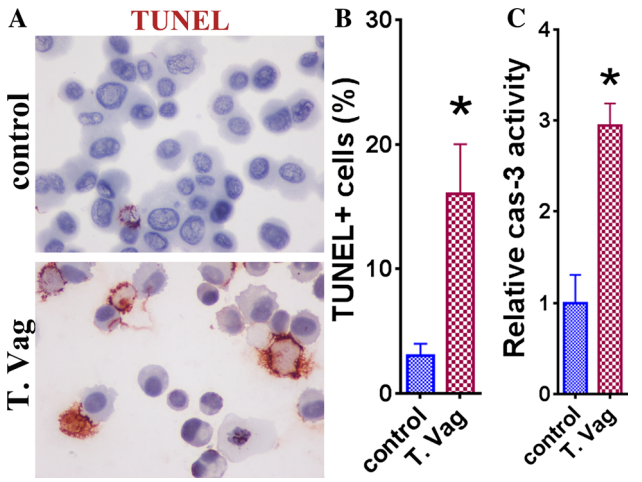

Fig. 4 Culture supernatant of *T. vag* promotes apoptosis of PC-3 cells. **a** Representative of TUNEL staining. **b** TUNEL + cells in 3–5 randomly selected high-power fields of three slides were counted. **c** Cellular caspase-3 activity was measured as described in methods. Results are expressed as mean activity relative to controls + SEM. Assays were done in triplicate. A significant difference in the percentage of TUNEL + cells or relative caspase-3 activity cells treated with culture supernatant of *T. vag* and those in controls is indicated by the *asterisk* ($p < 0.05$). Shown is representative of two independent experiments. Original magnification **a** ×400

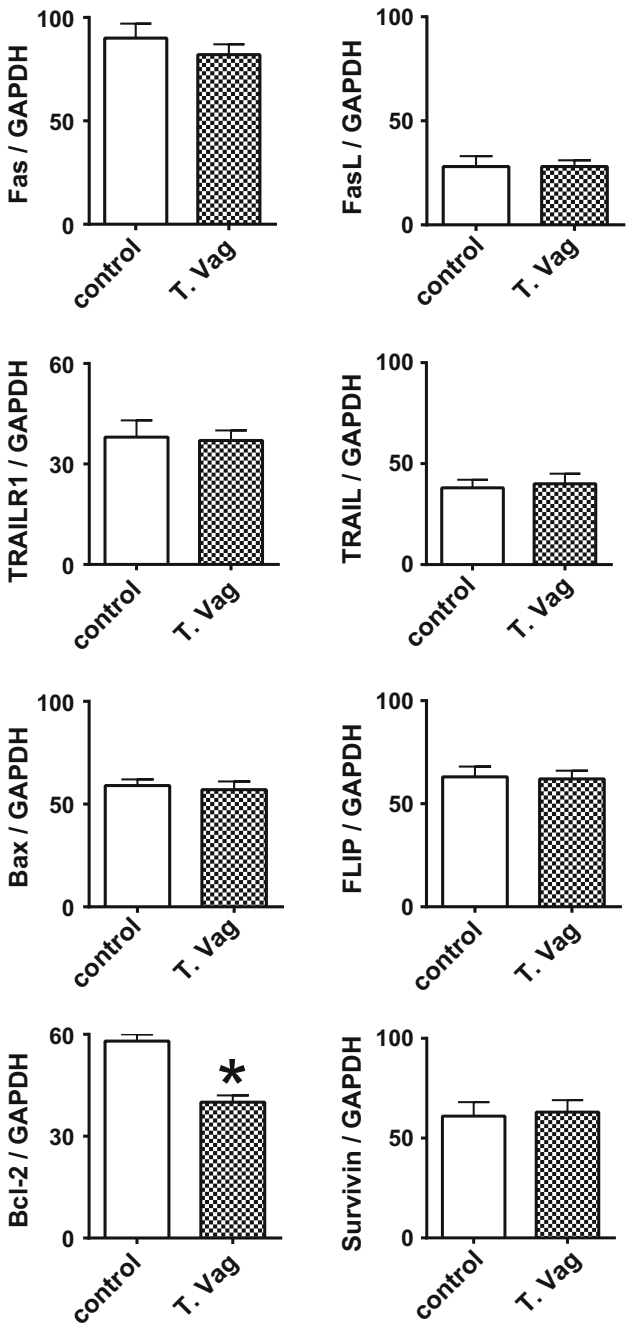

Fig. 5 Effect of culture supernatant of *T. vag* on expression of pro- and anti-apoptotic molecules in PC-3 cells evaluated by RT-PCR mRNA was extracted as described in the methods. Experiments are done in triplicate, and results are expressed as the mean ratio of pro- and anti-apoptotic molecule densitometric units/GAPDH + SEM (×100) and are representative of two independent experiments. A significant difference in mRNA expression between cells treated with culture supernatant of *T. vag* and those in controls is indicated by the *asterisk* ($p < 0.05$)

supernatant of *T. vag* correlated with increased expression of p21. Thus, our study indicated cultured supernatant of *T. vag* disrupted the balance between pro- and anti-

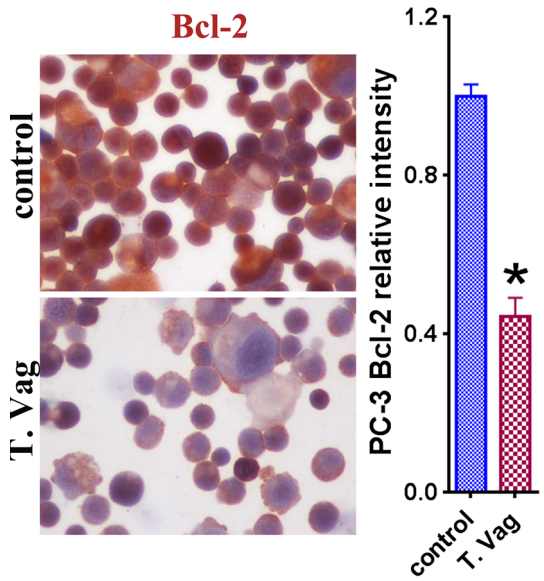

Fig. 6 Effect of culture supernatant of *T. vag* on expression of Bcl-2 in PC-3 cells evaluated by IHC Shown are representative pictures of IHC. The relative staining intensity in 3–5 randomly selected high-power fields of three slides from each group was analyzed by MetaMorph software. Results are expressed as the average integrated staining intensity of three slides + SEM relative to that in control cells. A significant difference in staining intensity between cells treated with culture supernatant of *T. vag* and those in controls is indicated by the *asterisk* ($p < 0.05$). Shown is representative of two independent experiments. Original magnification ×400

proliferative molecules by upregulation of anti-proliferative molecule p21 in PC-3 to inhibit cell proliferation in PCA cells. Upregulation of expression of p21 is also one of the mechanisms by which IL-9 inhibits growth of melanoma cells [36].

Apoptosis is also called programmed cell death. It is mediated by sequential activations of caspases [37, 38]. Pro- and anti-proliferative molecules are pivotal in cell apoptosis. On one hand, Fas, FasL, TRAIL-R1, TRAIL and Bax promote cell apoptosis, and thus, they are regarded as pro-apoptotic molecules. On the other hand, FLIP, Bcl-2 and survivin inhibit apoptosis, and accordingly, they are regarded as anti-apoptotic molecules [39]. In this study, we found that the pro-apoptotic effect of cultured supernatant of *T. vag* correlated with decreased expression of anti-apoptotic molecule Bcl-2. Thus, our study indicated cultured supernatant of *T. vag* disrupted the balance between pro- and anti-apoptotic molecules by downregulation of anti-apoptotic molecule Bcl-2 in PC-3 to induce apoptosis in PCA cells. Bcl-2 seems to be a critical anti-apoptotic molecule since its upregulation is one of the mechanisms by which IL-35 inhibits apoptosis in pancreatic cancer cells [40].

It might be necessary to point out that the expression of cyclin E was unexpectedly found to be increased in the group of cultured supernatant of *T. vag*. The detailed possible mechanisms are still under investigation. One possible explanation for this might be due to an adaptive response to cell injury to prevent cells from further damage. It might be also important to point out the fact that not all pro- and anti-proliferative as well as pro- and anti-apoptotic molecules were investigated in this study. It will not be a surprise for us at all that some other unexamined molecule might also contribute significantly to inhibition of proliferation and promotion of apoptosis in PC-3 prostate cancer cells treated with culture supernatant of *T. vag*. Nevertheless, our study might highlight that a seemly unwelcomed *T. vag* might be potentially beneficial to patients in an early neoplastic state.

In summary, culture supernatant of *T. vag* inhibits growth of prostate cancer by altering its balance between proliferation and apoptosis. Further study is warranted to elucidate the true role of *T. vag* in the pathogenesis of PCA.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA: Cancer J Clin. 2015;65(1):5–29.
2. Crawford ED. Epidemiology of prostate cancer. Urology. 2003;62(6):3–12.
3. Dirkx M, Boyer MP, Pradhan P, Brittingham A, Wilson WA. Expression and characterization of a beta-fructofuranosidase from the parasitic protist *Trichomonas vaginalis*. BMC Biochem. 2014;15(1):12.
4. Petrin D, Delgaty K, Bhatt R, Garber G. Clinical and microbiological aspects of *Trichomonas vaginalis*. Clin Microbiol Rev. 1998;11(2):300–17.
5. Satterwhite CL, Torrone E, Meites E, Dunne EF, Mahajan R, Ocfemia MCB, et al. Sexually transmitted infections among US women and men: prevalence and incidence estimates, 2008. Sex Transm Dis. 2013;40(3):187–93.
6. Workowski KA, Berman SM. Centers for disease control and prevention sexually transmitted disease treatment guidelines. Clin Infect Dis. 2011;53(Suppl 3):S59–63.
7. Wynder EL, Mabuchi K, Whitmore WF. Epidemiology of cancer of the prostate. Cancer. 1971;28(2):344–60.
8. Harkness A. Discussion on non-specific prostatitis. Proc Royal Soc Med. 1955;48(5):413–24.
9. Perl G, Schapira H, Ragazzoni H. Male urogenital trichomoniasis. J Mount Sinai Hospital, New York. 1965;32:495.
10. Gardner Jr WA, Culberson DE. Pathology of urogenital trichomoniasis in men. In: Trichomonads parasitic in humans. New York: Springer; 1990, p. 291–6.

11. Smith C, Gardner W Jr. Inflammation-proliferation: possible relationships in the prostate. Prog Clin Biol Res. 1986;239: 317–25.
12. Twu O, Dessí D, Vu A, Mercer F, Stevens GC, De Miguel N, et al. Trichomonas vaginalis homolog of macrophage migration inhibitory factor induces prostate cell growth, invasiveness, and inflammatory responses. Proc Natl Acad Sci. 2014;111(22): 8179–84.
13. Caini S, Gandini S, Dudas M, Bremer V, Severi E, Gherasim A. Sexually transmitted infections and prostate cancer risk: a systematic review and meta-analysis. Cancer Epidemiol. 2014;38(4):329–38. doi:10.1016/j.canep.2014.06.002.
14. Donders GG, Depuydt CE, Bogers J-P, Vereecken AJ. Association of *Trichomonas vaginalis* and cytological abnormalities of the cervix in low risk women. PLoS One. 2013;8(12):e86266.
15. Groom H, Warren AY, Neal DE, Bishop KN. No evidence for infection of UK prostate cancer patients with XMRV, BK virus, *Trichomonas vaginalis* or human papilloma viruses. PLoS One. 2012;7(3):e34221.
16. Alderete J, Pearlman E. Pathogenic *Trichomonas vaginalis* cytotoxicity to cell culture monolayers. Br J Vener Dis. 1984;60(2):99–105.
17. Gilbert R, Elia G, Beach D, Klaessig S, Singh B. Cytopathogenic effect of *Trichomonas vaginalis* on human vaginal epithelial cells cultured in vitro. Infect Immun. 2000;68(7):4200–6.
18. Guenthner PC, Secor WE, Dezzutti CS. *Trichomonas vaginalis*-induced epithelial monolayer disruption and human immunodeficiency virus type 1 (HIV-1) replication: implications for the sexual transmission of HIV-1. Infect Immun. 2005;73(7): 4155–60.
19. Rasmussen S, Nielsen M, Lind I, Rhodes J. Morphological studies of the cytotoxicity of *Trichomonas vaginalis* to normal human vaginal epithelial cells in vitro. Genitourin Med. 1986;62(4):240–6.
20. Rendón-Maldonado JG, Espinosa-Cantellano M, González-Robles A, Martinez-Palomo A. *Trichomonas vaginalis:* in vitrophagocytosis of lactobacilli, vaginal epithelial cells, leukocytes, and erythrocytes. Exp Parasitol. 1998;89(2):241–50.
21. Quan J-H, Kang B-H, Cha G-H, Zhou W, Koh Y-B, Yang J-B, et al. *Trichonomas vaginalis* metalloproteinase induces apoptosis of SiHa cells through disrupting the Mcl-1/Bim and Bcl-xL/Bim complexes. PLoS one. 2014;9(10):e110659.
22. Salvador-Membreve DMC, Jacinto SD, Rivera WL. *Trichomonas vaginalis* induces cytopathic effect on human lung alveolar basal carcinoma epithelial cell line A549. Exp Parasitol. 2014;147: 33–40.
23. Beal C, Goldsmith R, Kotby M, Sherif M, El-Tagi A, Farid A, et al. The plastic envelope method, a simplified technique for culture diagnosis of trichomoniasis. J Clin Microbiol. 1992;30(9):2265–8.
24. Nielsen TJ, Pradhan P, Brittingham A, Wilson WA. Glycogen accumulation and degradation by the trichomonads *Trichomonas vaginalis* and *Trichomonas* tenax. J Eukaryot Microbiol. 2012; 59(4):359–66.
25. Tan S, Singh M, Yap E, Ho L, Moe K, Howe J, et al. Colony formation of *Blastocystis hominis* in soft agar. Parasitol Res. 1996;82(4):375–7.
26. Fang Y, Bradley MJ, Cook KM, Herrick EJ, Nicholl MB. A potential role for resveratrol as a radiation sensitizer for melanoma treatment. J Surg Res. 2013;183(2):645–53.
27. Fang Y, DeMarco VG, Nicholl MB. Resveratrol enhances radiation sensitivity in prostate cancer by inhibiting cell proliferation and promoting cell senescence and apoptosis. Cancer Sci. 2012;103(6):1090–8.
28. Fang Y, Herrick EJ, Nicholl MB. A possible role for perforin and granzyme B in resveratrol-enhanced radiosensitivity of prostate cancer. J Androl. 2012;33(4):752–60.
29. Fang Y, Moore BJ, Bai Q, Cook KM, Herrick EJ, Nicholl MB. Hydrogen peroxide enhances radiation-induced apoptosis and inhibition of melanoma cell proliferation. Anticancer Res. 2013;33(5):1799–807.
30. Fang Y, Sharp GC, Yagita H, Braley-Mullen H. A critical role for TRAIL in resolution of granulomatous experimental autoimmune thyroiditis. J Pathol. 2008;216(4):505–13.
31. Fang Y, Wei Y, DeMarco V, Chen K, Sharp GC, Braley-Mullen H. Murine FLIP transgene expressed on thyroid epithelial cells promotes resolution of granulomatous experimental autoimmune thyroiditis in DBA/1 mice. Am J Pathol. 2007;170(3):875–87.
32. Johnson D, Walker C. Cyclins and cell cycle checkpoints. Annu Rev Pharmacol Toxicol. 1999;39(1):295–312.
33. Ohtsubo M, Theodoras AM, Schumacher J, Roberts JM, Pagano M. Human cyclin E, a nuclear protein essential for the G1-to-S phase transition. Mol Cell Biol. 1995;15(5):2612–24.
34. Sherr CJ. G1 phase progression: cycling on cue. Cell. 1994;79(4):551–5.
35. Sherr CJ. D-type cyclins. Trends Biochem Sci. 1995;20(5): 187–90.
36. Fang Y, Chen X, Bai Q, Qin C, Mohamud AO, Zhu Z, et al. IL-9 inhibits HTB-72 melanoma cell growth through upregulation of p21 and TRAIL. J Surg Oncol. 2015;111(8):969–74.
37. Griffith TS, Brunner T, Fletcher SM, Green DR, Ferguson TA. Fas ligand-induced apoptosis as a mechanism of immune privilege. Science. 1995;270(5239):1189–92.
38. Thompson CB. Apoptosis in the pathogenesis and treatment of disease. Science. 1995;267(5203):1456.
39. Fang Y, Braley-Mullen H. Cultured murine thyroid epithelial cells expressing transgenic Fas-associated death domain-like interleukin-1β converting enzyme inhibitory protein are protected from Fas-mediated apoptosis. Endocrinology. 2008;149(7): 3321–9.
40. Nicholl MB, Ledgewood CL, Chen X, Bai Q, Qin C, Cook KM, et al. Il-35 promotes pancreas cancer growth through enhancement of proliferation and inhibition of apoptosis: evidence for a role as an autocrine growth factor. Cytokine. 2014;70(2):126–33.