Assessment of histopathological parameters and survival difference in EBV-positive and EBV-negative prostate carcinoma

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Research Article

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Abstract

Introduction: Several studies have shown an association between prostate carcinoma (PCa) and certain viral infections, such as HPV, EBV, CMV, etc. Despite the evidence about the presence of the EBV in PCa tissues, it is unknown if the presence of EBV is associated with any distinct histopathological characteristics and/or survival advantages/disadvantages in patients with PCa. In this study, therefore, we analyzed the LMP-1 expression, and histopathological characteristics of EBV-positive and -negative PCa tissues, followed by the survival analysis in patients from the two groups.

Material & Methods: LMP-1 expression was determined using immunohistochemistry in the EBV PCR-positive FFPE PCa samples. Subsequently, two key parameters for the characterization of PCa, i.e., Gleason scores and perineural invasion (PNI), along with intratumoral lymphocytes and stromal lymphocytic infiltration was studied in the EBV-positive and -negative PCa tissues. Lastly, the survival benefit analysis of EBV-positive and EBV-negative PCa patients was performed.

Results: EBV LMP1 protein expression was found in 70.96% of EBV PCR-positive PCa tissues. Histopathological analysis showed significantly higher ($p<0.05$) mean major and total Gleason scores, and perineural invasion (80%) in EBV-positive as compared with the EBV-negative PCa samples. We also found a higher percentage of intratumoral and tumor-stromal lymphocytic infiltration in EBV-positive PC samples as compared to EBV-negative PCa samples. Overall, the survival proportion was similar in both EBV-positive and EBV-negative groups. However, a small difference in survival benefit emerged in the 38th month, where the mean percent survival was higher in EBV-positive PCa (41.6%) patients as compared to EBV-negative PCa patients (19.96%).

Conclusion: In conclusion, the presence of EBV in the PCa tissues may lead to aggressive forms of cancer. Further studies with a larger sample size are required to strengthen the link between EBV, PCa prognosis, and survival.

Introduction

Despite being one of the most common carcinomas in men worldwide (1), the etiology and events related to the onset and progression of prostate carcinoma (PCa) remain poorly understood (2). Several studies have shown an association between PCa and certain viral infections, such as HPV, EBV, CMV, BK virus, and SV40 (3–9). Epstein-Barr virus (EBV) has been found in the PCa specimens (3, 4), however, the role of EBV in either onset or progression of PCa is not well characterized (5). Despite the evidence about the presence of the EBV in prostate carcinoma tissues, it is not known whether the presence of EBV in PCa tissue is associated with any distinct histopathological characteristics in the PCa tissues and/or survival advantages/disadvantages in patients with PCa.

We have recently shown and characterized EBV in PCa tissues (10). In this study, we report the immunohistochemical (IHC) analysis of the EBV LMP1 expression on the prostate carcinoma tissue

samples, followed by the analysis of the differences in the histopathological characteristics, and survival of the patients with EBV-positive and EBV-negative PCa.

Materials & Methods

Samples collection

This study is a sequel to our previous study, where we reported PCR-based EBV-positivity in 39/99 FFPE prostate carcinoma (PCa) samples, followed by characterization of EBV-latency associated genes expression (11). In this study, we determined LMP-1 expression using IHC in the EBV PCR positive tissues followed by the differential analysis of key histopathological features of EBV positive (PCR and LMP-1) and negative PCa samples. The study was approved by the Aga Khan University Ethics Review Committee (AKU-ERC #: 2021-1460-18525).

Immunohistochemical detection of EBV LMP1 protein in prostate carcinoma tissues

For this analysis, 31/39 EBV PCR-positive PCa samples were used for immunohistochemical expression analysis of EBV LMP1 oncoprotein, while eight samples were dropped due to insufficient tissue. The formalin-fixed tissue sections were deparaffinized and rehydrated. Antigen retrieval was enhanced by revealing epitopes with the use of citrate buffer at pH 9.0. Subsequently, the sections were stained with mouse antibody anti-EBV LMP1 (CS1-4) (Dako, Agilent Technologies Denmark). The detection of LMP1 has been carried out through staining with a Horseradish peroxidase-labeled anti-mouse secondary antibody, while Diaminobenzidine (Dako DAB) was used as substrate chromogen, and hematoxylin was used as a counterstain. The slides were further processed using Autostainer Link 48 (Dako Agilent) following the manufacturer's instructions. The slides showing either the membranous or the cytoplasmic staining (brown color) of tumor cells in the specimens were considered positive for LMP1. The slides were scored using the following criteria described by *Mao et al*. (12): score 4 = 81–100% LMP1 positivity, score 3 = 51–80% LMP1 positivity, score 2 = 11–50% LMP1 positivity, and score 1 = 0–10% LMP1 positivity in the cells. Furthermore, the intensity of immunostaining was scored 0 for negative, while 1, 2, and 3 for, respectively, weak, moderate, to strong immunostaining.

Histopathological characterization of EBV + ve and EBV-ve PCa samples

The EBV-positive and EBV-negative PCa samples were graded by an experienced histopathologist using the WHO 2016/ ISUP 2014-based prostate cancer grading system (13). All the PCa samples were diagnosed as prostatic adenocarcinoma of acinar type. In this study, two of the most important parameters for the characterization of PCa, i.e. Gleason scores (major, minor, and total) and perineural invasion (PNI) (14, 15), along with the presence of intra-tumoral lymphocytes, stromal lymphocytes, and benign tissue lymphocytic infiltration was noted, and used for the analysis for the quantitative parameters such as mean, standard deviation, and the standard error of the mean; whereas for the

qualitative data, the counts and percentages were used. To characterize the presence of lymphocytic infiltration in the tissue, criteria in solid tumors by the International Immuno-Oncology Biomarker Working Group (16) were used with the modification where the intensity of lymphocytic infiltration was rated using a scale of 0 to + 3; with 0 being no lymphocytes observed, whereas + 1, +2, + 3 indicated the presence of 1–15, 16–25, and > 25 lymphocytes, respectively. On light microscopy, the PCa samples were scanned for the presence of the lymphocytes and were categorized into the intratumoral, stroma, and adjacent benign tissue infiltration in the specimens. Subsequently, Spearman correlation analysis was performed to examine the association between different histopathological parameters, described above, in the two groups. The analysis was performed using GraphPad Prism 8.4 and in all analyses, $p < 0.5$ was considered statistically significant.

Survival benefit analysis in EBV-positive and EBV-negative PCa groups

For survival benefit analysis, the 99 PCa positive (including both EBV-positive and -negative groups) patients or next of kin were contacted over the phone. Out of 99 patients, only 74 patients or their next of kin responded. Hence, the survival analysis was performed for 74 patients for a period of 38 months, starting February 2019 (date of sample collection) till February 2022, using Logrank (Mantel-Cox) test (17).

Results

IHC analysis of EBV LMP1 protein expression in EBV PCR-positive PCa samples

Immunohistochemical staining of EBV PCR-positive PCa samples showed focal cytoplasmic granular LMP1 expression in 70.96% (22/31) PCa tissues (Fig. 1). All LMP1 positive PCa samples had an intensity score of 1–2, with weak to moderate immunostaining (Fig. 1).

EBV status of the prostate carcinoma samples and its correlation to histopathological characteristics

The analysis of the Gleason scores (major, minor, and total) showed that the EBV-positive PCa samples had significantly higher mean major (4.12 ± 0.64; $p < 0.05$) and total (8.24 ± 1.22 $p < 0.05$) Gleason scores as compared with the EBV-negative PCa samples (major: 3.84 ± 0.58; total: 7.75 ± 1.03). Descriptive analysis for distribution of perineural invasion in EBV-positive and EBV-negative PCa samples showed that a higher percentage of EBV-positive PCa (80%) had perineural invasion as compared to EBV-negative PCa (67.3%) samples (Fig. 2). This finding was further supported by correlation analysis, which showed a moderate positive correlation ($r = 0.47$; $p < 0.05$) between Gleason score (total) and perineural invasion only in EBV-positive PCa tissues (Table 2).

Table 2

Correlation between histopathological parameters in EBV-positive and -negative PCa samples: The table shows the correlation coefficients observed between different histopathological parameters (Gleason (major, minor, and total) scores and perineural invasion) in EBV-positive and EBV-negative PCa samples. The r values found to be statistically significant ($p < 0.05$) are shown in bold.

Variables	EBV + Gleason major	EBV + Gleason minor	EBV + Gleason total	EBV + Perineural invasion
EBV + Gleason major	—	**0.46**	**0.86**	0.33
EBV + Gleason minor	**0.46**	—	**0.85**	**0.47**
EBV + Gleason total	**0.86**	**0.85**	—	**0.47**
EBV + perineural invasion	**0.33**	**0.47**	**0.47**	—
Variables	**EBV- Gleason major**	**EBV-Gleason minor**	**EBV-Gleason total**	**EBV-Perineural invasion**
EBV- Gleason major	—	**0.50**	**0.81**	**0.28**
EBV-Gleason minor	**0.50**	—	**0.90**	0.07
EBV-Gleason total	**0.81**	**0.90**	—	0.20
EBV-Perineural invasion	**0.28**	**0.07**	0.20	—

Furthermore, the analysis of lymphocytic infiltration showed tumor-stromal lymphocytic infiltration in 74.07% of EBV-positive and 66.67% in EBV-negative PCa tissues (Figs. 3A and 3B). Similarly, intratumoral lymphocytic infiltration was observed in 33.34% of EBV-positive and 31.11% of EBV-negative PCa tissues (Figs. 3C and 3D). Additionally, 92.59% of EBV-positive PCa samples showed lymphocytic infiltration in the benign tissue adjacent to the tumor cells, as compared to 80% of EBV-negative PCa tissue samples (Figs. 3E and 3F).

Survival analysis of EBV-positive and EBV-negative prostate cancer group

For EBV-positive and EBV-negative prostate carcinoma groups, the percent survival over 38 months was calculated using Logrank (Mantel-Cox test). Overall, the survival proportion was similar in both EBV-positive and EBV-negative groups (Fig. 4). However, a small difference in survival benefit emerged in the

38th month, where the mean percent survival was higher in EBV-positive PCa (41.6%) patients as compared to EBV-negative PCa patients (19.96%) ($p = 0.69$). The month and year-wise distribution of deaths in EBV-positive and EBV-negative PCa samples are described in Supplementary Fig. 1.

Discussion

Here we studied LMP-1 expression, and histopathological characteristics of the EBV-positive and -negative PCa tissues, followed by survival analysis of the patients from the two groups

Descriptive and association analysis showed that the mean major and total Gleason scores in EBV-positive PCa samples were higher as compared to EBV-negative PCa samples, and a positive association between Gleason scores (total). This finding suggests an association between EBV infection in the prostate cancer tissues and the higher total Gleason scores. This observation was corroborated by an analysis of PNI distribution, where a higher percentage of EBV-positive PCa (80%) had PNI as compared to EBV-negative PCa samples (67.3%), and EBV status was positively correlated with PNI, further strengthening the possibility that EBV infection might be associated with the aggressive forms of cancer. These findings are clinically important as Gleason score and PNI are associated with an aggressive form of PCa (18–21). Although in literature, nothing is known about EBV and its relationship with PCa, however, it has been reported that the presence of EBV in nasopharyngeal carcinoma is associated with the progression of cancer since it involves multiple hallmarks of cancer (22, 23). Clinically, EBV-positive nasopharyngeal carcinoma tends to be more invasive with increased metastasis as compared with EBV-negative nasopharyngeal carcinoma cases (24). At the same time, EBV-positive nasopharyngeal carcinoma cases are more responsive to radiotherapy and chemotherapy (25) as compared to EBV-negative NPC cases.

Next, LMP1 expression was determined in PCa samples. Although, for EBV detection, an RNA in situ hybridization (ISH) is the test of the choice (26), however, IHC-based detection of LMP1 is commonly performed especially in low and middle-income countries (27, 28). Some studies have reported that the rates of detection between IHC LMP and ISH are comparable (29, 30). We found LMP1 expression in 70.96% of the cases. To the best of our knowledge, IHC-based expression of EBV LMP1 in PCa tissues has been reported for the first time in this study. Among EBV-associated epithelial cancers, the IHC-based expression of EBV LMP1 is better characterized in EBV-associated nasopharyngeal carcinoma and has been observed in variable percentages in different studies, ranging from 40–86% (27, 31–35).

In this report, we have found higher percentage of intratumoral lymphocytic infiltration, tumor stromal lymphocytic infiltration and the lymphocytic infiltration in the benign tissue adjacent to the tumor tissue in EBV-positive PC samples as compared to EBV-negative PCa samples. These findings seem to support earlier reported studies where EBV-positive nasopharyngeal carcinoma samples had a significantly higher number of tumor-infiltrating lymphocytes as compared with EBV-negative nasopharyngeal carcinoma samples (36), and the presence of a higher number of tumor-infiltrating lymphocytes is associated with better survival (37). Similarly, better survival is associated with the presence of tumor-infiltrating

lymphocytes in EBV-associated gastric carcinoma (38). Tumor-infiltrating lymphocytes have an important role in prostate carcinoma (39), however, further studies are required to establish the role of EBV-associated tumor-infiltrating lymphocytes in prostate carcinoma.

Lastly, a survival benefit analysis in EBV-positive and EBV-negative patients with PCa was performed. Overall, the EBV status was independent of survival proportions, however, a survival advantage was observed in the 38th month, where the EBV-positive PCa group was found to have a higher survival as compared to the EBV-negative group. Previous studies have suggested a conflicting role of EBV in cancer; Song *et al* showed that the presence of EBV conferred a survival advantage to the patients with EBV-associated gastric carcinoma (40), whereas in EBV-associated nonkeratinizing subtype of nasopharyngeal carcinoma, EBV infection has been associated with increased risk of distant metastasis (41, 42). The role of EBV in the progression of prostate carcinoma has not been previously reported. As indicated earlier, the enhanced survival may be attributed to increased lymphocytic infiltration, as is observed in EBV-associated nasopharyngeal and gastric carcinoma (37, 38). There are certain limitations with respect to the survival analysis. Firstly, other clinical parameters, such as treatment regimens during the period of this analysis could not be included as this information was not available for biopsies. Secondly, there was an imbalance in the number of EBV-positive (n = 25) and EBV-negative retrospectively collected PCa samples (n = 49), which might introduce some sampling bias (43). However, this bias may be limited as events (deaths) were considered and not the actual sample number in the analysis. Finally, the survival analysis was limited to 38 months (the current month) only. Analysis beyond 38 months may provide additional information on survival benefits.

In conclusion, this study provides evidence that the presence of EBV in the prostate carcinoma tissues may lead to aggressive forms of PCa. Further studies with a larger sample size are required to strengthen the link between EBV, PCa prognosis, and survival.

Declarations

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Conflict of interest: None to declare

Author's contribution:

Conceptualization: SHA; Methodology: KA, AS, SF, GH, FA; First draft: KA; Final draft review, and supervision: KG, NM, SHA

Data availability: All data generated or analyzed during this study are included in this published article (and its supplementary information files).

References

1. Gandaglia G, Leni R, Bray F, Fleshner N, Freedland SJ, Kibel A, et al. Epidemiology and prevention of prostate cancer. European urology oncology. 2021.
2. Phua TJ. The etiology and pathophysiology genesis of benign prostatic hyperplasia and prostate cancer: a new perspective. Medicines. 2021;8(6):30.
3. Whitaker NJ, Glenn WK, Sahrudin A, Orde MM, Delprado W, Lawson JS. Human papillomavirus and Epstein Barr virus in prostate cancer: Koilocytes indicate potential oncogenic influences of human papillomavirus in prostate cancer. The Prostate. 2013;73(3):236–41.
4. Nahand JS, Khanaliha K, Mirzaei H, Moghoofei M, Baghi HB, Esghaei M, et al. Possible role of HPV/EBV coinfection in anoikis resistance and development in prostate cancer. BMC cancer. 2021;21(1):1–19.
5. Abidi SH, Bilwani F, Ghias K, Abbas F. Viral etiology of prostate cancer: genetic alterations and immune response. A literature review. International Journal of Surgery. 2018;52:136–40.
6. Banerjee S, Alwine JC, Wei Z, Tian T, Shih N, Sperling C, et al. Microbiome signatures in prostate cancer. Carcinogenesis. 2019;40(6):749–64.
7. Martinez-Fierro ML, Leach RJ, Gomez-Guerra LS, Garza-Guajardo R, Johnson-Pais T, Beuten J, et al. Identification of viral infections in the prostate and evaluation of their association with cancer. BMC cancer. 2010;10(1):1–7.
8. Sfanos KS, Sauvageot J, Fedor HL, Dick JD, De Marzo AM, Isaacs WB. A molecular analysis of prokaryotic and viral DNA sequences in prostate tissue from patients with prostate cancer indicates the presence of multiple and diverse microorganisms. The Prostate. 2008;68(3):306–20.
9. Chen Y, Wei J. Identification of pathogen signatures in prostate cancer using RNA-seq. PLoS One. 2015;10(6):e0128955.
10. Khalid A, Alisalman S, Saira F, Ghulam H, Kulsoom G, Farhat A, et al. Detection and Characterization of Latency Stage of EBV and Histopathological Analysis of Prostatic Adenocarcinoma Tissues. Scientific Reports. 2022.
11. Khalid A, Ali S, Saira F, Ghulam H, Kulsoom G, Farhat A, et al. Detection and Characterization of Latency Stage of EBV and Histopathological Analysis of Prostatic Adenocarcinoma Tissues. Scientific Reports. 2022.
12. Mao Y, Zhang D-W, Zhu H, Lin H, Xiong L, Cao Q, et al. LMP1 and LMP2A are potential prognostic markers of extranodal NK/T-cell lymphoma, nasal type (ENKTL). Diagnostic pathology. 2012;7(1):1–8.
13. Humphrey PA, Moch H, Cubilla AL, Ulbright TM, Reuter VE. The 2016 WHO Classification of Tumours of the Urinary System and Male Genital Organs-Part B: Prostate and Bladder Tumours. European urology. 2016;70(1):106–19.
14. Rusthoven CG, Carlson JA, Waxweiler TV, Yeh N, Raben D, Flaig TW, et al., editors. The prognostic significance of Gleason scores in metastatic prostate cancer. Urologic Oncology: Seminars and Original Investigations; 2014: Elsevier.

15. Zhang L-j, Wu B, Zha Z-l, Qu W, Zhao H, Yuan J, et al. Perineural invasion as an independent predictor of biochemical recurrence in prostate cancer following radical prostatectomy or radiotherapy: a systematic review and meta-analysis. BMC urology. 2018;18(1):1–10.
16. Hendry S, Salgado R, Gevaert T, Russell PA, John T, Thapa B, et al. Assessing tumor infiltrating lymphocytes in solid tumors: a practical review for pathologists and proposal for a standardized method from the International Immuno-Oncology Biomarkers Working Group: Part 2: TILs in melanoma, gastrointestinal tract carcinomas, non-small cell lung carcinoma and mesothelioma, endometrial and ovarian carcinomas, squamous cell carcinoma of the head and neck, genitourinary carcinomas, and primary brain tumors. Advances in anatomic pathology. 2017;24(6):311.
17. Cox DR, Oakes D. Analysis of survival data: Chapman and Hall/CRC; 2018.
18. Herman C, Kattan M, Ohori M, Scardino P, Wheeler T. Primary Gleason pattern as a predictor of disease progression in gleason score 7 prostate cancer: a multivariate analysis of 823 men treated with radical prostatectomy. The American journal of surgical pathology. 2001;25(5):657–60.
19. Sæter T, Bogaard M, Vlatkovic L, Waaler G, Servoll E, Nesland JM, et al. The relationship between perineural invasion, tumor grade, reactive stroma and prostate cancer-specific mortality: A clinicopathologic study on a population-based cohort. The Prostate. 2016;76(2):207–14.
20. Zareba P, Flavin R, Isikbay M, Rider JR, Gerke TA, Finn S, et al. Perineural invasion and risk of lethal prostate cancer. Cancer Epidemiology and Prevention Biomarkers. 2017;26(5):719–26.
21. Lee IH, Roberts R, Shah RB, Wojno KJ, Wei JT, Sandler HM. Perineural invasion is a marker for pathologically advanced disease in localized prostate cancer. International Journal of Radiation Oncology* Biology* Physics. 2007;68(4):1059–64.
22. Wong KC, Hui EP, Lo K-W, Lam WKJ, Johnson D, Li L, et al. Nasopharyngeal carcinoma: an evolving paradigm. Nature Reviews Clinical Oncology. 2021;18(11):679–95.
23. Yin H, Qu J, Peng Q, Gan R. Molecular mechanisms of EBV-driven cell cycle progression and oncogenesis. Medical microbiology and immunology. 2019;208(5):573–83.
24. Nakanishi Y, Wakisaka N, Kondo S, Endo K, Sugimoto H, Hatano M, et al. Progression of understanding for the role of Epstein-Barr virus and management of nasopharyngeal carcinoma. Cancer and Metastasis Reviews. 2017;36(3):435–47.
25. Yoshizaki T, Ito M, Murono S, Wakisaka N, Kondo S, Endo K. Current understanding and management of nasopharyngeal carcinoma. Auris Nasus Larynx. 2012;39(2):137–44.
26. Abusalah MAH, Gan SH, Al-Hatamleh MA, Irekeola AA, Shueb RH, Yean Yean C. Recent advances in diagnostic approaches for epstein–barr virus. Pathogens. 2020;9(3):226.
27. Omoseebi O, Akinde O, Obadofin OO, Ogun G, Banjo AA. Association of Epstein–Barr virus (EBV) with malignancy of the nasopharynx in Lagos, Nigeria. Annals of Tropical Pathology. 2017;8(1):29.
28. Habeeb R, Monem F. EBV Plasma Epstein-Barr Virus (EBV) DNA as a Biomarker for Diagnosis of EBV-positive Hodgkin Lymphoma in Syria. The Journal of Infection in Developing Countries. 2021;15(12):1917–22.

29. Qi Z-L, Han X-Q, Hu J, Wang G-H, Gao J-W, Wang X, et al. Comparison of three methods for the detection of Epstein-Barr virus in Hodgkin's lymphoma in paraffin-embedded tissues. Molecular Medicine Reports. 2013;7(1):89–92.
30. Kurniawan AN, Kodariah R, Elisabeth M, Roezin A, Gondhowiardjo S. Evaluation of EBV-LMP1 as prognostic indicator of nasopharyngeal carcinoma in Indonesian patients. Medical Journal of Indonesia. 2002;11(2):81–6.
31. Rosales-Pérez S, Cano-Valdez AM, Flores-Balcázar CH, Guedea-Edo F, Lino-Silva LS, Lozano-Borbalas A, et al. Expression of Epstein-Barr virus-encoded latent membrane protein (LMP-1), p16 and p53 proteins in nonendemic nasopharyngeal carcinoma (NPC): a clinicopathological study. Archives of medical research. 2014;45(3):229–36.
32. Salano VE, Mwakigonja AR, Abdulshakoor A, Kahinga AA, Richard EM. Epstein-Barr Virus Latent Membrane Protein-1 Expression in Nasopharyngeal Carcinoma. JCO Global Oncology. 2021;7:1406–12.
33. Ruuskanen M, Irjala H, Minn H, Vahlberg T, Randen-Brady R, Hagström J, et al. Epstein-Barr virus and human papillomaviruses as favorable prognostic factors in nasopharyngeal carcinoma: a nationwide study in Finland. Head & Neck. 2019;41(2):349–57.
34. Yates SM, Iliyasu Y, Ahmed SA, Liman AA. Immunohistochemical expression of epstein–Barr virus Latent Membrane Protein-1 in nasopharyngeal carcinoma. Annals of Tropical Pathology. 2017;8(2):99.
35. Vera-Sempere F, Burgos J, Botella M, Cordoba J, Gobernado M. Immunohistochemical expression of Epstein-Barr virus-encoded latent membrane protein (LMP-1) in paraffin sections of EBV-associated nasopharyngeal carcinoma in Spanish patients. European Journal of Cancer Part B: Oral Oncology. 1996;32(3):163–8.
36. Ooft ML, van Ipenburg JA, Braunius WW, Zuur CI, Koljenović S, Willems SM. Prognostic role of tumor infiltrating lymphocytes in EBV positive and EBV negative nasopharyngeal carcinoma. Oral oncology. 2017;71:16–25.
37. Yang L, Liu G, Li Y, Pan Y. The emergence of tumor-infiltrating lymphocytes in nasopharyngeal carcinoma: Predictive value and immunotherapy implications. Genes & Diseases. 2021.
38. Kang B, Seo A, Yoon S, Bae H, Jeon S, Kwon O, et al. Prognostic value of tumor-infiltrating lymphocytes in Epstein–Barr virus-associated gastric cancer. Annals of Oncology. 2016;27(3):494–501.
39. Yunger S, Bar El A, Zeltzer L-a, Fridman E, Raviv G, Laufer M, et al. Tumor-infiltrating lymphocytes from human prostate tumors reveal anti-tumor reactivity and potential for adoptive cell therapy. Oncoimmunology. 2019;8(12):e1672494.
40. Song HJ, Srivastava A, Lee J, Kim YS, Kim KM, Kang WK, et al. Host inflammatory response predicts survival of patients with Epstein-Barr virus–associated gastric carcinoma. Gastroenterology. 2010;139(1):84–92. e2.

41. Lee A, Ma B, Ng WT, Chan A. Management of nasopharyngeal carcinoma: current practice and future perspective. J Clin Oncol. 2015;33(29):3356–64.
42. Zhao C-X, Zhu W, Ba Z-Q, Xu H-J, Liu W-D, Zhu B, et al. The regulatory network of nasopharyngeal carcinoma metastasis with a focus on EBV, lncRNAs and miRNAs. American journal of cancer research. 2018;8(11):2185.
43. Dickman PW, Adami HO. Interpreting trends in cancer patient survival. Journal of internal medicine. 2006;260(2):103–17.

Figures

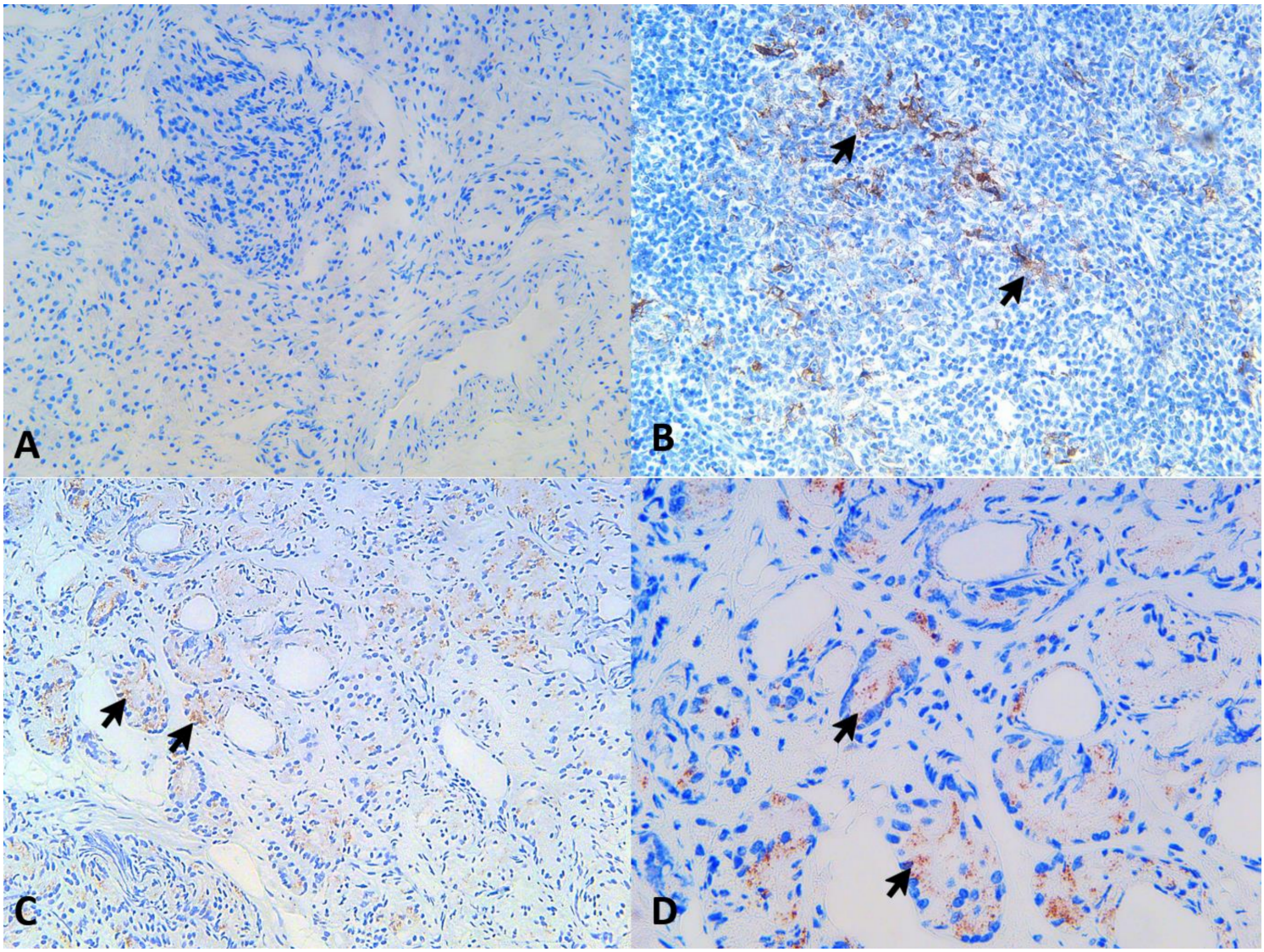

Figure 1

Immunohistochemical staining of LMP1 in PCa tissue samples. A) LMP1 negative PCa tissue sample. **B)** Positive control: EBV-associated nasopharyngeal carcinoma sample with confirmed EBV LMP1 expression (black arrows) **C-D)** PCa tissue samples showing weak-moderate EBV LMP1 staining (black

arrows) in the tumor cells. The presence of granular cytoplasmic LMP1 immunostaining was labeled as positive. Original magnification: 200X in A, B, and C and 400X in D.

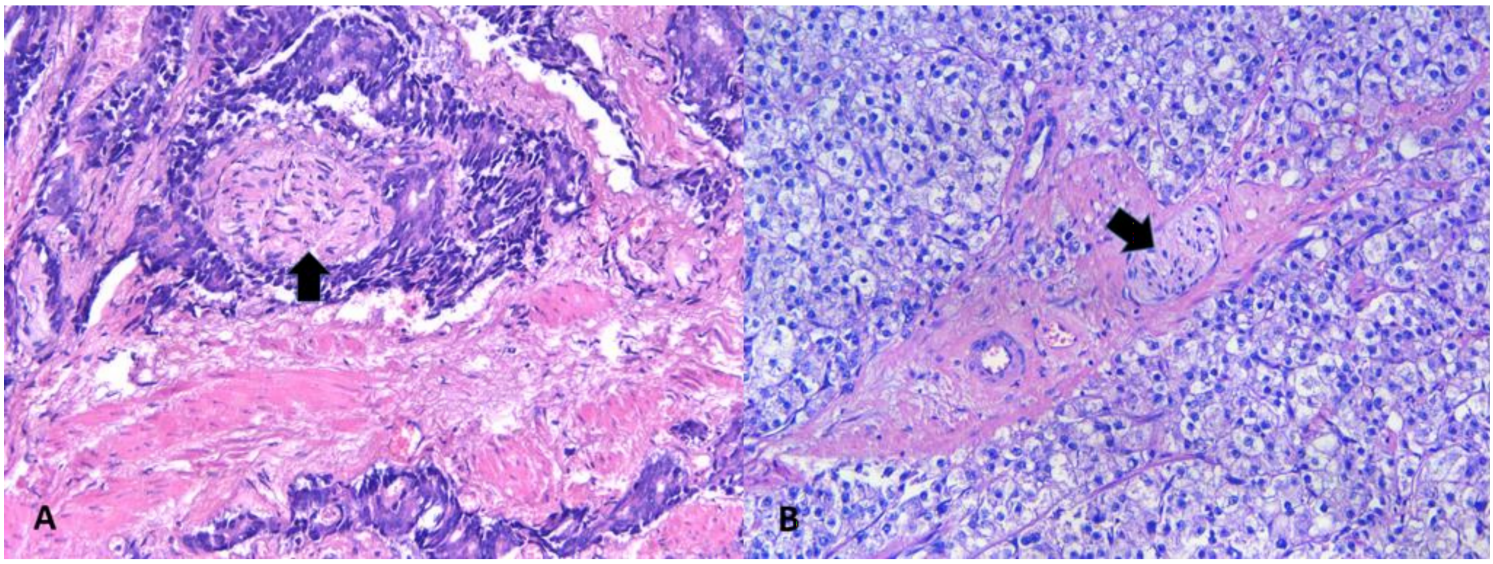

Figure 2

Representative histopathological images of EBV-positive and EBV-negative PCa with present and absent PNI, respectively. A) representative histopathological images of EBV-positive PCa, Gleason score of 9 (4+5), showing the crowded cribriform glands infiltrating the perineurium of the nerve in the center, indicating the presence of perineural invasion (PNI) (marked by black arrow). (H&E; original magnification: 200X). **B)** histopathological image of EBV-negative PCa, Gleason score of 8 (4+4), showing the poorly formed glands with hypernephroid cells. The center shows an un-involved nerve (PNI is absent) marked with an arrowhead (H&E; original magnification: 200X).

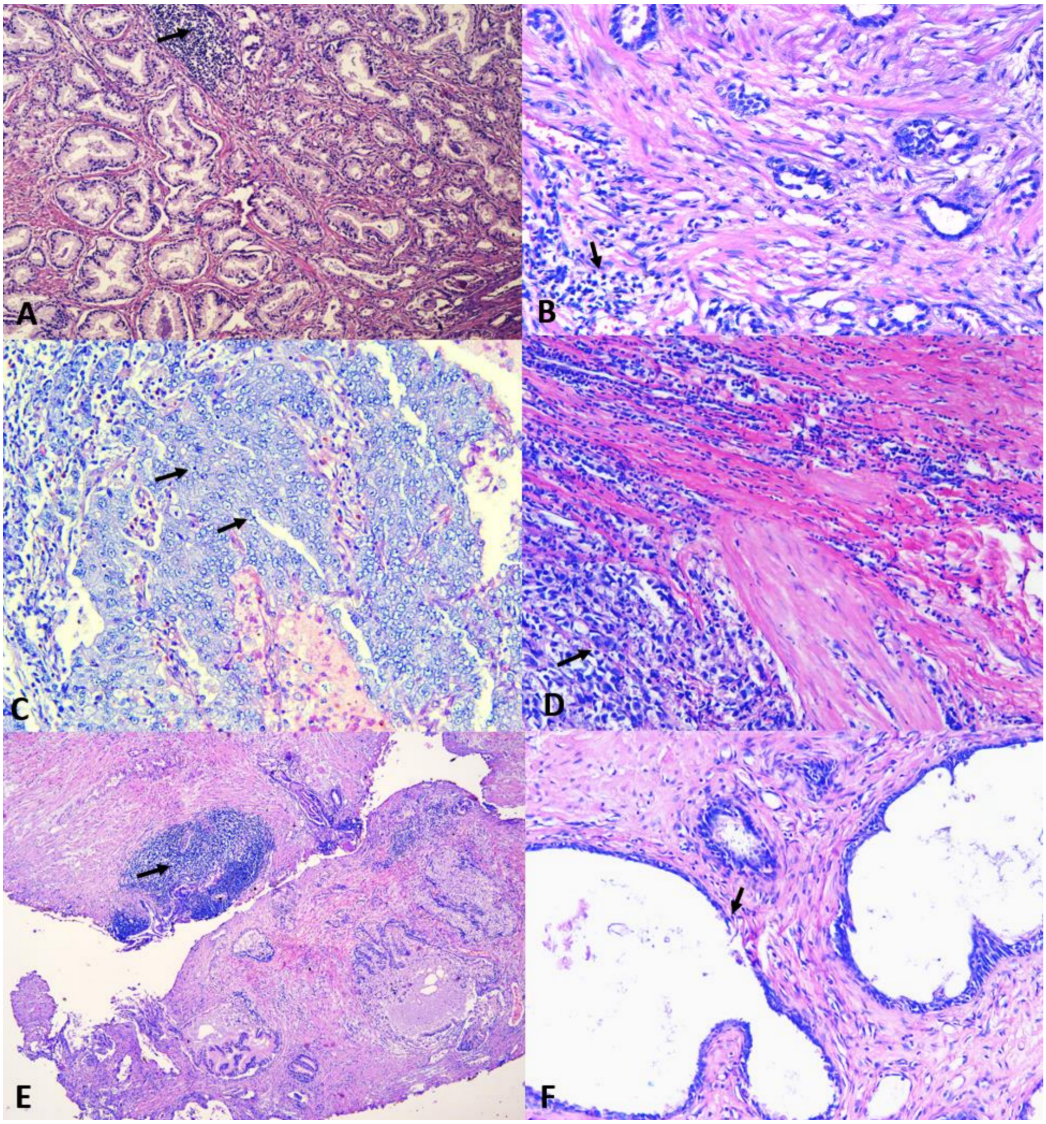

Figure 3

Lymphocytic infiltration in EBV-positive and EBV-negative PCa tissues: Histological images showing lymphocytic infiltration (shown with black arrowheads) in **A)** EBV-positive tissue with tumor stromal lymphocytic infiltration (3+) and Gleason score 3+3 (Hematoxylin and Eosin (H&E); original magnification: 100X), **B)** EBV-negative sample with tumor stromal lymphocytic infiltration (1+) and Gleason score (4+4) (H&E; 200X), **C)** EBV-positive sample with intratumoral lymphocytic infiltration (1+) and Gleason score (5+5) (H&E; original magnification: 200X), **D)** EBV-negative sample with intratumoral lymphocytic infiltration (1+) and Gleason score (5+5) (H&E; original magnification: 200x), **E)** EBV-positive sample with lymphocytic infiltration in the adjacent benign tissue (3+) (H&E; original magnification: 40X) **F)** EBV-negative sample with lymphocytic infiltration in the adjacent benign tissue (1+) (H&E; original magnification: 200X)

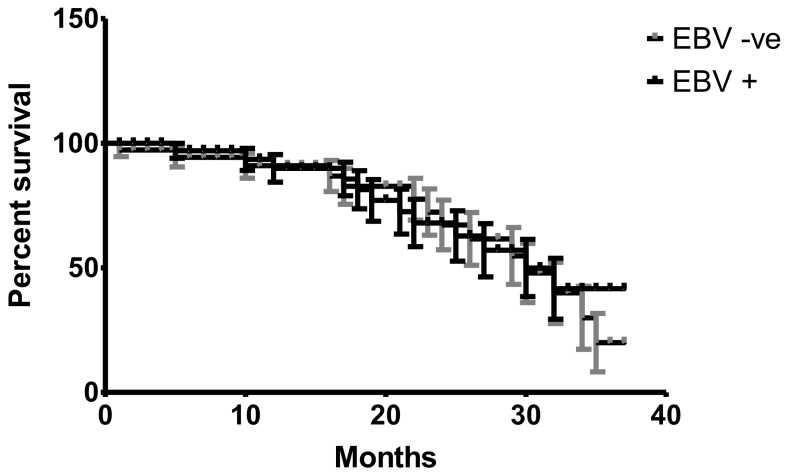

Figure 4

The percent survival analysis of EBV-positive and EBV-negative PCa patients. The x-axis shows percent survival, while the y-axis shows months considered in this analysis, for both EBV-positive and -negative PCa groups.

Supplementary Files

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- FigS1.tiff