HISTOPATHOLOGICAL PATTERNS OF LYMPHOVASCULAR AND PERINEURAL INVASION IN VIRUS-ASSOCIATED RECTAL CANCER

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Abstract

Background: Rectal adenocarcinoma remains a major oncological challenge because of its potential for local recurrence, regional and distant dissemination, and cancer-related mortality. In recent years, the potential involvement of viral agents in colorectal tumorigenesis has attracted growing scientific interest. Human papillomavirus (HPV), Epstein–Barr virus (EBV), and human cytomegalovirus (HCMV) have been reported in colorectal neoplastic tissues; however, the biological relevance of these findings and their association with tumor invasiveness have not been fully elucidated.

Objective: To characterize the histopathological patterns of lymphovascular invasion (LVI) and perineural invasion (PNI) in virus-associated rectal adenocarcinoma and assess their relationships with tumor differentiation, depth of local invasion, lymph node involvement, metastatic potential, and prognostic indicators.

Materials and Methods: The study is planned to encompass surgical and biopsy specimens from patients diagnosed with rectal adenocarcinoma between 2016 and 2026. Conventional histopathological examination will be supplemented by immunohistochemical, molecular-genetic, and morphometric analyses. Viral

status, particularly HPV positivity, will be determined using appropriate diagnostic assays. Lymphovascular invasion will be assessed using CD31, CD34, ERG, and D2-40, whereas perineural invasion will be examined using S100, SOX10, and PGP9.5. Additional markers, including E-cadherin, N-cadherin, vimentin, MMP-2, MMP-9, Ki-67, and p53, will be employed to evaluate tumor differentiation, proliferative activity, and features associated with invasive progression.

Expected Results: The study is expected to identify potential differences in the prevalence and histomorphological patterns of LVI and PNI between virus-positive and virus-negative rectal adenocarcinomas. The relationships between viral status, tumor differentiation, TNM stage, lymph node involvement, invasive characteristics, and prognostic parameters will also be explored.

Conclusion: An integrated histopathological, immunohistochemical, molecular-genetic, and morphometric investigation may provide further insight into the mechanisms underlying tumor invasion and progression in rectal adenocarcinoma. The findings may contribute to the development of an enhanced pathomorphological framework for prognostic assessment.

Keywords: rectal adenocarcinoma; human papillomavirus; HPV; Epstein–Barr virus; EBV; cytomegalovirus;lymphovascular invasion; perineural invasion; pathomorphology; immunohistochemistry; tumorprogression; metastasis; prognosis.

1.Introduction

Rectal cancer represents a significant global health problem and remains one of the major causes ofcancer-related morbidity and mortality. Despite improvements in surgery, chemotherapy,radiotherapy, and molecular diagnostics, a substantial proportion of patients continue to developlocal recurrence or distant metastasis. The biological behavior of rectal cancer is determined bygenetic and epigenetic alterations, tumor microenvironment, immune response, and

morphologicalcharacteristics.

Among the morphological parameters, lymphovascular invasion and perineural invasion areimportant indicators of aggressive tumor behavior. LVI refers to the presence of tumor cells withinlymphatic or blood vessel spaces and represents an important step in the metastatic cascade. PNI ischaracterized by invasion or spread of malignant cells along or around peripheral nerves and isassociated with local recurrence and unfavorable survival outcomes.

Increasing attention has also been directed toward infectious agents in colorectal carcinogenesis. HPV, EBV, and HCMV have been detected in colorectal tumor tissues. HPV16 and HPV18 are high-riskgenotypes whose E6 and E7 oncoproteins can interfere with p53 and retinoblastoma proteinpathways, affecting cell-cycle regulation, apoptosis, genomic stability, epithelial-mesenchymaltransition, and cellular migration.

Recent evidence has strengthened the hypothesis of an association between HPV infection andcolorectal cancer. A 2026 systematic review and meta-analysis including 20 case-control studiesreported a pooled odds ratio of approximately 2.39 for the association between HPV infection andcolorectal cancer, with a stronger association reported in Asian populations. Nevertheless,heterogeneity in viral genotypes, geographic regions, biological materials, and diagnostic methodslimits definitive causal interpretation.

EBV has also been investigated as a potential factor in colorectal carcinogenesis. Viral proteins mayinfluence proliferation, survival, immune regulation, epithelial-mesenchymal transition, andmigration. A 2023 systematic review and meta-analysis reported an overall EBV prevalence ofapproximately 18% among colorectal cancer cases and suggested an association with colorectalcancer. However, the direct causal role remains uncertain. HCMV has attracted attention because of its possible influence on the tumor microenvironment andimmune response. Some studies have reported associations

between HCMV-positive colorectaltumors and unfavorable outcomes, but the precise mechanisms remain to be clarified.

A major unresolved question is whether viral infection is associated with the invasive phenotype ofrectal adenocarcinoma. Although many studies have examined viral prevalence, relatively few havespecifically investigated whether virus-positive tumors demonstrate different patterns of LVI and PNIcompared with virus-negative tumors. Therefore, integrated investigation of viral status, LVI, PNI,tumor differentiation, tumor budding, lymph node metastasis, TNM stage, and molecular markers isjustified.

2. Aim of the Study

The aim of this study is to comprehensively investigate the pathomorphological characteristics oflymphovascular and perineural invasion in virus-associated rectal cancer, determine theirrelationships with biological tumor characteristics, local extension, metastatic potential, and clinicalprognosis, and develop an improved pathomorphological prognostic algorithm based onimmunohistochemical and molecular-genetic parameters.

3. Objectives

1. To analyze age, sex, tumor localization, histological type, differentiation grade, and TNM stage.

2. To determine viral infection, particularly HPV, using molecular-genetic and immunohistochemicalmethods.

3. To determine the frequency and morphological characteristics of LVI.

4. To investigate the morphological characteristics of PNI and tumor–nerve interactions.5. To assess CD31, CD34, ERG, D2-40, S100, SOX10, and PGP9.5 as diagnostic markers.6. To investigate invasive and progression mechanisms using E-cadherin, N-cadherin, vimentin,MMP-2, MMP-9, Ki-67, and p53.

7. To determine relationships between LVI, PNI, lymph node metastasis, TNM stage, differentiation,and other clinicopathological parameters.

8. To compare the prognostic significance of LVI and PNI between virus-positive and virus-negativegroups.

9. To develop an integrated pathomorphological-prognostic algorithm.

4. Materials and Methods

The planned study will include pathological specimens obtained from patients with rectal cancerduring 2016–2026. Surgical resection specimens and biopsy samples stored in pathology andoncology archives, as well as newly obtained materials, will be evaluated. Standard hematoxylin and eosin staining will be used for histological examination. Tumor type, differentiation, depth ofinvasion, tumor budding, lymph node status, and other pathological parameters will be recorded.

LVI will be assessed using CD31, CD34, ERG, and D2-40. These markers facilitate identification anddifferentiation of blood and lymphatic vascular structures and may reveal small or equivocal foci ofinvasion. PNI will be assessed using S100, SOX10, and PGP9.5 to improve visualization of neuralstructures and subtle perineural invasion.

Molecular and immunohistochemical assessment will focus primarily on HPV; EBV and HCMV may beassessed according to the final study design and availability of diagnostic methods. E-cadherin,N-cadherin, vimentin, MMP-2, MMP-9, Ki-67, and p53 will be used to characterizeepithelial-mesenchymal transition, extracellular matrix remodeling, proliferative activity, and tumorprogression.

Morphometric assessment will include quantitative evaluation of selected parameters, including theextent of vascular and neural invasion, tumor budding, and immunohistochemical expression.Statistical analysis will compare virus-positive and virus-negative groups and assess associationswith TNM stage, differentiation, lymph node metastasis, and survival-related parameters.

5. Scientific Novelty

The novelty of the study consists in an integrated investigation of virus-associated rectaladenocarcinoma with particular emphasis on LVI and PNI. Virus-positive and virus-negative tumorswill be compared to identify relationships between viral infection, tumor differentiation, histologicalstructure, and invasive growth. LVI and PNI will be assessed with a panel of vascular, lymphatic, andneural markers, allowing more objective identification of diagnostically difficult invasion.

The study will also investigate the relationship between viral infection and molecular characteristicsof invasive tumor growth. The expression of E-cadherin, N-cadherin, vimentin, MMP-2, MMP-9, Ki-67, and p53 may help explain the biological mechanisms underlying aggressive tumor behavior. Clinical,morphological, immunohistochemical, molecular, and morphometric parameters will be integratedinto a prognostic model.

6. Expected Results

It is expected that virus-positive and virus-negative rectal adenocarcinomas will demonstratedifferences in the frequency and severity of LVI and PNI. These invasion patterns may be associatedwith higher TNM stage, lower differentiation, increased tumor budding, lymph node metastasis, andpotentially distant metastatic disease.

Immunohistochemistry is expected to improve detection of LVI and PNI compared with routine H&E;examination alone. D2-40 may improve identification of lymphatic invasion, while CD31, CD34, andERG may facilitate vascular assessment. S100, SOX10, and PGP9.5 may improve visualization ofneural structures.

Tumors with more pronounced invasive characteristics may demonstrate molecular featuresconsistent with epithelial-mesenchymal transition and extracellular matrix remodeling. ReducedE-cadherin with increased N-cadherin and vimentin may indicate a more invasive phenotype, whileincreased MMP-2 and MMP-9 may reflect enhanced extracellular matrix degradation.

7. Practical Significance

The practical importance of this study lies in improving pathological assessment and prognosticstratification of patients with rectal cancer. Accurate identification of LVI and PNI is important forestimating metastatic potential, and appropriate immunohistochemical markers may improvedetection in diagnostically difficult cases.

Comparison of virus-positive and virus-negative tumors may provide additional informationregarding biological heterogeneity. An integrated algorithm incorporating clinical, morphological,immunohistochemical, molecular-genetic, and morphometric parameters may help pathologists andoncologists identify patients at increased risk of lymph node metastasis, distant progression, and 4 unfavorable prognosis.

8. Discussion

The relationship between viral infection and colorectal cancer remains an active area of research. Detection of viral nucleic acids or antigens does not necessarily prove causality; viral infection mayrepresent a pathogenetic factor, a passenger infection, or an association with a particular tumormicroenvironment. Therefore, investigation should move beyond simple viral detection towardcharacterization of morphological and molecular tumor phenotypes.
LVI and PNI are valuable morphological endpoints because they represent mechanisms throughwhich malignant cells overcome tissue barriers and gain access to anatomical pathways facilitatingdissemination. Viral proteins can influence cell adhesion, proliferation, apoptosis,epithelial-mesenchymal transition, and extracellular matrix remodeling, all of which may contributeto vascular invasion.

PNI involves complex interactions between malignant cells and the neural microenvironment.Neurotrophic and adhesion signaling systems such as NGF/TrkA, BDNF/TrkB, GDNF/GFRα, NCAM, andL1CAM have been implicated

in colorectal cancer PNI. Therefore, combining viral profiling withassessment of vascular, lymphatic, neural, epithelial, mesenchymal, and proliferative markers mayprovide a more comprehensive understanding of invasive tumor behavior.

9. Conclusion

Virus-associated rectal cancer is a promising field at the intersection of oncovirology, pathologicalanatomy, molecular pathology, and tumor biology. Although existing studies suggest possibleassociations between HPV, EBV, HCMV, and colorectal cancer, the relationship between viralinfection and specific invasive morphological features remains insufficiently characterized. Comprehensive assessment of LVI and PNI in virus-positive and virus-negative rectaladenocarcinomas may clarify the relationship between viral status and aggressive tumor phenotype.Integration of histomorphology, immunohistochemistry, molecular-genetic testing, and digitalmorphometry may improve identification of invasive features and provide additional prognosticinformation. An integrated pathomorphological-prognostic algorithm may contribute to improvedrisk stratification and individualized assessment of patients.

References

1. Damin DC, Ziegelmann PK, Damin AP. Human papillomavirus infection and colorectal cancer risk:a meta-analysis. Colorectal Disease. 2013;15:e428.

2. Ibragimova MK, Tsyganov MM, Litviakov NV. Human papillomavirus and colorectal cancer. MedicalOncology. 2018;35(11):140.

3. Baandrup L, Thomsen LT, Olesen TB, et al. The prevalence of human papillomavirus in colorectalcancer and adenoma: a meta-analysis. European Journal of Cancer. 2014;50(13):2212–2220.4. Yang M, Huo Y, Huang Y, et al. Human papillomavirus infection and prevalence of colorectalcancer: an updated systematic review and meta-analysis of global data. International Journal of

Surgery. 2026;112(1):1815–1825.

5. Jafari Maskouni E, Jamalvandi T, Tabatabaei F, et al. Association between Epstein–Barr virus andcolorectal cancer: a systematic review and meta-analysis. Microbial Pathogenesis.
2023;179:106087.

6. Koch A, et al. Involvement of HHV-4 (Epstein–Barr Virus) and HHV-5 (Cytomegalovirus) inInflammatory Bowel Disease and Colorectal Cancer: A Meta-Analysis. Cancers. 2022;14(20):5085.7. Lee JH, Jang HS, Kim JG, et al. Lymphovascular invasion is a significant prognosticator in rectalcancer patients who receive preoperative chemoradiotherapy followed by total mesorectal excision.Annals of Surgical Oncology. 2012;19(4):1213–1221.

8. Nikberg M, Chabok A, Leto R, et al. Lymphovascular and perineural invasion have a prognosticimpact in stage II rectal cancer. Acta Oncologica.
2016;55(12):1418–1424.

9. Knijn N, Mogk SC, Teerenstra S, Simmer F, Nagtegaal ID. Perineural invasion is a strong prognosticfactor in colorectal cancer: a systematic review. American Journal of Surgical Pathology.
2016;40(1):103–112.

10. Yang Y, Wang G, He J, et al. Prognostic value of perineural invasion in colorectal cancer: ameta-analysis. Journal of Gastrointestinal Surgery.
2015;19(6):1113–1122.

11. van Wyk HC, et al. The role of perineural invasion in predicting survival in patients with primaryoperable colorectal cancer: A systematic review. Critical Reviews in Oncology/Hematology.
2017;112:11–20.

12. Fukuda Y, et al. S100-stained perineural invasion is associated with poor prognosis in stage I/IIcolorectal cancer. Pathology International. 2022;72(2):117–127.

13. Chen T, et al. The incidence and prognosis value of perineural invasion in rectal carcinoma: frommeta-analyses and real-world clinical pathological features. Clinical Oncology. 2023;35(10):e621.14. Wang H, et al. Perineural invasion in colorectal cancer: mechanisms of action and clinicalrelevance. Cellular Oncology. 2024;47(1):1–17.