PATHOMORPHOLOGICAL CHARACTERISTICS OF LYMPHOVASCULAR AND PERINEURAL INVASION IN VIRUS-ASSOCIATED RECTAL CARCINOMA

O.Kh. Mirzamukhamedov

Scientific adviser: ***D.A. Nishanov****, DSc, Professor*

Department of Pathological Anatomy, Tashkent State Medical University,

Tashkent, Uzbekistan

Abstract

Background: Rectal cancer is one of the most important problems in modern oncology due to its high incidence, risk of local recurrence, distant metastasis, and cancer-related mortality. Increasing attention has recently been paid to the possible role of viral infections in colorectal carcinogenesis. Human papillomavirus (HPV), Epstein–Barr virus (EBV), and human cytomegalovirus (HCMV) have been detected in colorectal tumor tissues in a number of studies. However, their pathogenetic significance and association with invasive tumor behavior remain insufficiently understood.

Objective: To investigate the pathomorphological features of lymphovascular invasion (LVI) and perineural invasion (PNI) in virus-associated rectal cancer and determine their relationships with tumor differentiation, local extension, lymph node metastasis, metastatic potential, and prognosis.

Materials and Methods: The planned study will include surgical and biopsy specimens obtained from patients with rectal adenocarcinoma treated during 2016–2026. Histomorphological examination will be combined with immunohistochemical and molecular-genetic methods. Viral infection, particularly HPV infection, will be assessed using modern diagnostic techniques. LVI will be evaluated using CD31, CD34, ERG, and D2-40, while PNI will be investigated using

S100, SOX10, and PGP9.5. E-cadherin, N-cadherin, vimentin, MMP-2, MMP-9, Ki-67, and p53 will be used to characterize tumor progression and invasive phenotype.

Expected Results: Virus-positive and virus-negative rectal adenocarcinomas are expected to demonstrate differences in the frequency and morphological characteristics of LVI and PNI. Associations between viral status, invasive phenotype, tumor differentiation, TNM stage, lymph node metastasis, and prognostic parameters are expected to be identified.

Conclusion: A comprehensive pathomorphological, immunohistochemical, molecular-genetic, and morphometric assessment of virus-associated rectal cancer may improve understanding of tumor invasion and progression and provide a basis for an integrated prognostic algorithm.

Keywords

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

Introduction

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

Among the morphological parameters, lymphovascular invasion and perineural invasion are important indicators of aggressive tumor behavior. LVI refers to the presence of tumor cells within lymphatic or blood vessel spaces and represents an

important step in the metastatic cascade. PNI is characterized by invasion or spread of malignant cells along or around peripheral nerves and is associated 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-risk genotypes whose E6 and E7 oncoproteins can interfere with p53 and retinoblastoma protein pathways, affecting cell-cycle regulation, apoptosis, genomic stability, epithelial-mesenchymal transition, and cellular migration.

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

EBV has also been investigated as a potential factor in colorectal carcinogenesis. Viral proteins may influence proliferation, survival, immune regulation, epithelial-mesenchymal transition, and migration. A 2023 systematic review and meta-analysis reported an overall EBV prevalence of approximately 18% among colorectal cancer cases and suggested an association with colorectal cancer. However, the direct causal role remains uncertain.

HCMV has attracted attention because of its possible influence on the tumor microenvironment and immune response. Some studies have reported associations between HCMV-positive colorectal tumors 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 of rectal adenocarcinoma. Although many studies have

examined viral prevalence, relatively few have specifically investigated whether virus-positive tumors demonstrate different patterns of LVI and PNI compared with virus-negative tumors. Therefore, integrated investigation of viral status, LVI, PNI, tumor differentiation, tumor budding, lymph node metastasis, TNM stage, and molecular markers is justified.

Aim of the Study

The aim of this study is to comprehensively investigate the pathomorphological characteristics of lymphovascular and perineural invasion in virus-associated rectal cancer, determine their relationships with biological tumor characteristics, local extension, metastatic potential, and clinical prognosis, and develop an improved pathomorphological prognostic algorithm based on immunohistochemical and molecular-genetic parameters.

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.

Materials and Methods

The planned study will include pathological specimens obtained from patients with rectal cancer during 2016–2026. Surgical resection specimens and biopsy samples stored in pathology and oncology 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 of invasion, 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 and differentiation of blood and lymphatic vascular structures and may reveal small or equivocal foci of invasion. PNI will be assessed using S100, SOX10, and PGP9.5 to improve visualization of neural structures and subtle perineural invasion.

Molecular and immunohistochemical assessment will focus primarily on HPV; EBV and HCMV may be assessed 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 characterize epithelial-mesenchymal transition, extracellular matrix remodeling, proliferative activity, and tumor progression.

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

Scientific Novelty

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

The study will also investigate the relationship between viral infection and molecular characteristics of 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 integrated into a prognostic model.

Expected Results

It is expected that virus-positive and virus-negative rectal adenocarcinomas will demonstrate differences in the frequency and severity of LVI and PNI. These invasion patterns may be associated with higher TNM stage, lower differentiation, increased tumor budding, lymph node metastasis, and potentially 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, and ERG may facilitate vascular assessment. S100, SOX10, and PGP9.5 may improve visualization of neural structures.

Tumors with more pronounced invasive characteristics may demonstrate molecular features consistent with epithelial-mesenchymal transition and extracellular matrix remodeling. Reduced E-cadherin with increased N-cadherin and

vimentin may indicate a more invasive phenotype, while increased MMP-2 and MMP-9 may reflect enhanced extracellular matrix degradation.

Practical Significance

The practical importance of this study lies in improving pathological assessment and prognostic stratification of patients with rectal cancer. Accurate identification of LVI and PNI is important for estimating metastatic potential, and appropriate immunohistochemical markers may improve detection in diagnostically difficult cases.

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

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 may represent a pathogenetic factor, a passenger infection, or an association with a particular tumor microenvironment. Therefore, investigation should move beyond simple viral detection toward characterization of morphological and molecular tumor phenotypes.

LVI and PNI are valuable morphological endpoints because they represent mechanisms through which malignant cells overcome tissue barriers and gain access to anatomical pathways facilitating dissemination. Viral proteins can influence cell adhesion, proliferation, apoptosis, epithelial-mesenchymal transition, and extracellular matrix remodeling, all of which may contribute to 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, and L1CAM have been implicated in colorectal cancer PNI. Therefore, combining viral profiling with assessment of vascular, lymphatic, neural, epithelial, mesenchymal, and proliferative markers may provide a more comprehensive understanding of invasive tumor behavior.

Conclusion

Virus-associated rectal cancer is a promising field at the intersection of oncovirology, pathological anatomy, molecular pathology, and tumor biology. Although existing studies suggest possible associations between HPV, EBV, HCMV, and colorectal cancer, the relationship between viral infection and specific invasive morphological features remains insufficiently characterized.

Comprehensive assessment of LVI and PNI in virus-positive and virus-negative rectal adenocarcinomas may clarify the relationship between viral status and aggressive tumor phenotype. Integration of histomorphology, immunohistochemistry, molecular-genetic testing, and digital morphometry may improve identification of invasive features and provide additional prognostic information. An integrated pathomorphological-prognostic algorithm may contribute to improved risk 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.