EBV persistence in gastric cancer cases conventionally classified as EBER-ISH negative

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Abstract

The Epstein-Barr virus (EBV) causes various B-cell lymphomas and epithelial malignancies, including gastric cancer (GC) at frequencies ranging from 5-10% in adenocarcinomas (ADK) to 80% in GC with lymphoid stroma (GCLS). Using high-sensitivity methods, we recently detected EBV traces in a large cohort of EBV-negative B-cell lymphomas, suggesting a *hit-and-run* mechanism. Here, we used routine and higher-sensitivity methods [droplet digital PCR (ddPCR) for EBV segments on microdissected tumour cells and RNAscope for *EBNA1* mRNA] to assess EBV infection in a cohort of 40 GCs (28 ADK and 12 GCSL). ddPCR documented the presence of EBV nucleic acids in rare tumour cells of several cases conventionally classified as EBV-negative (ADK, 8/26; GCSL, 6/7). Similarly, RNAscope confirmed EBNA1 expression in rare tumour cells (ADK, 4/26; GCSL, 3/7). Finally, since EBV induces epigenetically changes that are heritable and retained after complete loss of the virus from the host cell, we studied the methylation pattern of EBV-specifically methylated genes (*Timp2*, *Eya1*) as a mark of previous EBV infection. Cases with EBV traces showed a considerable level of methylation in *Timp2* and *Eya1* genes that was similar to that observed in EBER-ISH positive cases and greater than cases not featuring any EBV traces. These findings suggest that: a) EBV may contribute to gastric pathogenesis more widely than currently acknowledged and b) indicate the methylation changes as a mechanistic framework for how EBV can act in a *hit-and-run* manner. Finally, we found that the viral state was of prognostic significance at univariate and multivariate analysis.