For all malignancies, not just those that are virally induced, a clearer understanding is needed of the switch during malignancy from a quiescent, dormant state to an active, progressing disease. Hence, we and others believe that to improve patient outcomes substantially, better noninvasive biomarkers are needed for more effective detection in cancer screening, monitoring of response to therapy, and prediction of relapse. In viral malignancies, the interplay between the virus and the tumor is likely to play a pivotal role in this switch and may provide potential biomarkers.

Cell-free DNA (cfDNA), referred to by the authors of an article in this issue of *The Journal of Infectious Diseases* as a liquid tumor biopsy, was first described 60 years ago [1] and has often been proposed as a cancer biomarker. Elevated levels of cfDNA are seen in cancer [2, 3] in part due to reduced deoxyribonuclease activity [4]. Because cancers are characterized by impaired apoptosis, lengths of cfDNA may differ in cancer compared to other states, and, indeed, its presence is believed to reflect tumor stage and underlying biologic proliferation. Elevated levels of cfDNA in plasma have been suggested for the diagnosis of certain cancers, but elevated levels of cfDNA are sometimes observed in benign disease [5]; therefore, levels of cfDNA are not tumor-specific markers. Importantly, however, patterns in cfDNA (eg, mutations, microsatellites, loss of heterozygosity) have the potential to provide tumor-specific markers and have been more widely investigated [6]. Further, methylation or hypermethylation, as measured by the presence of CpG islands, may have greater sensitivity and specificity for given clinical situations, including the potential aforementioned “switch.”

In the current report by Shamay et al [7], the presence of CpG methylation was used as a marker for cell-derived DNA, versus virion DNA, as detected by binding to MBD2-beads. In individuals with AIDS Kaposi sarcoma (KS), their data suggest that DNA sequences detected in plasma are indeed virion DNA and not tumor-DNA derived. Why this might be the case is the subject of speculation, and whether DNA in plasma has a function or is an epiphenomenological by-product of other processes is unclear. For example, cfDNA may function as a transposon-like infectious element, although this is harder to imagine in the setting of nonvirally induced malignancy.

In Herpesviridae-associated oncogenesis, the relative importance of latent and lytic infection is often finely balanced, as defined by the seminal experiments of George Klein and Eva Klein [8]. In Epstein–Barr virus (EBV) infection, their laboratories described viral nuclear antigens that are expressed during latency and discovered several chemicals that triggered the switch from latency to the lytic viral cycle, including the demethylating agent azacitidine, which is used as an anticancer agent in certain myelodysplastic syndromes. For example, the lytic or productive phase of EBV’s life cycle is induced by the expression of the viral BZLF1 gene in latently infected cells. The BZLF1 protein is a transactivator, which selectively binds to 2 classes of distinct DNA sequence motifs [9]. One class is similar to the motifs that are bound by members of the AP-1 transcription factor family to which BZLF1 belongs. The second class, which contains CpG motifs of relevance here, is predominant in viral promoters of early lytic genes, and is BZLF1’s preferred or exclusive target sequence when methylated. The BZLF1 gene is transiently expressed in newly infected B cells but fails to induce EBV’s lytic cycle potentially because the virion DNA is unmethylated. Recently, it has been found that the lack of 5-methylcytosine residues in CpG sites of virion DNA prevents the expression of essential lytic genes indispensable for viral DNA amplification during
productive infection [10]. This finding indicates that BZLF1 transactivates these promoters in a methylation-dependent fashion and explains how progeny virus synthesis is abrogated in newly infected B cells. In aggregate here, EBV has evolved to appropriate its host's mode of DNA methylation for its own epigenetic regulation, and this is likely to be the case for Kaposi sarcoma herpesvirus (KSHV) as well.

The well-documented expression patterns of herpesvirus genes contribute to the range of malignant histologies observed. In KSHV, many of the proposed viral oncogenic products are lytic genes [11]; hence, their contribution to oncogenesis, unlike the case with EBV, has often been questioned. Similarly, the major KSHV latent genes appear to coordinate molecular piracy and immune evasion, including microRNAs that down-regulated Toll-like receptors [12]. In KS lesions, only a small fraction, perhaps <1%, of cells appear to harbor lytic KSHV, whereas the majority of KSHV appears latent in spindle cells [13] and, indeed, the KSHV burden per cell is lower than in primary effusion lymphoma.

What does the Shamay et al [7] article then tell us about the pathogenesis of KSHV-related cancers? Different mechanisms may be involved; is this reflective of treatment strategies or are we still playing catch-up with the viruses? The low copy number and latency in KS suggests that antiviral drugs that predominantly target replicative lytic virus are unlikely to be effective, and studies of cidofovir and other anti-herpesvirus drugs have largely confirmed this by their lack of durable efficacy. On the other hand, approaches that rely on enhancing immune function and restore adaptive immune responses against KSHV are associated with regression of KS. Moreover, it appears that the immunogenic proteins in KSHV are early lytic antigens [14, 15].

Are there clinical applications of these findings? Could the methylation-dependent measurement of KSHV be used to differentiate active KS versus lymphatic damage in KS-related lymphedema as the authors perhaps suggest? Could the assay be useful in differentiating KS and multicentric Castleman disease (MCD)? (Previous studies have suggested that higher plasma viral loads of KSHV are associated with MCD, and indeed, that a rising level predicts relapse [16–20]). Prospective trials or cohort studies larger than the one presented here would be ideally required. However, the differentiation at lower titers between KS and MCD may be less clear cut. If, as one would suspect from the findings in primary effusion lymphoma, MCD is associated with elevated levels of cell-derived methylated KSHV DNA and, as described, KS is associated with high levels of circulating unmethylated virion-derived KSHV DNA, this technique could be used to aid differentiation of the 2 clinical entities, which require different clinical management strategies. The aim ultimately will be to prospectively identify the switch, from latent to lytic, but more relevantly from “carriers” to those developing cancer, in whom treatment strategies are clear. As the epigenome or, more specifically, the global methylome is sequenced and unraveled [21], the contribution of viruses should not be forgotten.

Note

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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