Biochemical Approaches to Detection of Epstein-Barr Virus in Human Tumors

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Summary

The application of biochemical studies for the detection of Epstein-Barr virus (EBV)-DNA in human tumor cells is discussed. These studies resulted in the consistent demonstration of viral nucleic acid in African Burkitt's lymphoma biopsies and in epithelial tumor cells of nasopharyngeal carcinomas. The viral DNA resides within those cells regularly in multiple copies per cell. Besides these tumors our group detected significant concentrations of EBV-DNA in a German lymphoma patient revealing histological characteristics of Burkitt's lymphoma. Moreover, virus DNA was also found in a patient suffering from immunoblastic lymphadenopathy. More than 50 additional B-cell lymphomas and more than 40 biopsies from patients with Hodgkin's disease did not contain detectable amounts of EBV-DNA when tested by nucleic acid hybridization. A tentative scheme of EBV-induced pathogenesis is discussed.

The consistent presence of EBV nucleic acid within tumor cells of 2 human cancers, African BL and NPC (5, 13, 14) appears to represent the first and as yet the only successful biochemical demonstration of a defined oncogenic agent in specific human tumor cells. It provides us with a base line for experimental approaches as well as for speculations on how to proceed in the further search for the role of viruses in human cancers.

In the following I will try to discuss the relevance of biochemical studies for establishing the etiology of specific human tumors by EBV. The consistent association of this virus with African BL and with NPC appears to be firmly established. EBV-DNA or virus-specific antigens have been demonstrated in more than 80 BL biopsies up to now (5, 6, 11–13). In these tumor patients in particular, serological evidence does not suffice in order to trace the etiological relationship of the agent to the disease. Besides BL and NPC, a number of patients with other lymphoproliferative diseases (like Hodgkin's disease or chronic lymphatic leukemia) show highly elevated EBV-antibody titers (1, 2, 4). In addition, even healthy control persons occasionally reveal exceptionally high titers.

Assuming a relationship of potential human tumor viruses to their transformed host cells similar to that observed in animal systems, we have to expect the presence of the viral genome or of substantial (and measurable) proportions thereof in every tumor cell. This postulate should be emphasized since we do not yet know of any exceptions in animal systems. The African BL, revealing usually multiple copies of EBV-DNA per tumor cell, clearly fits into this pattern. In this tumor the presence of EBV genomes can now also be demonstrated by the Epstein-Barr nuclear antigen test of Reedman and Klein (7), which permits the detection of virus-specific antigens in virtually all nuclei of those tumor cells. Although this represents a very convenient test system, it does not permit quantitative conclusions concerning the number of genome equivalents per cell.

Among all African cases tested, 2 showed exceptional features, i.e., they contained neither Epstein-Barr nuclear antigen nor detectable concentrations of EBV-DNA, despite a more or less typical histology and the presence of antibodies to EBV antigens in the serum of the respective patients (G. Klein, personal communication). Moreover, all of the American "BL's" tested thus far by nucleic acid hybridizations turned out to be negative, even when assayed under very sensitive conditions (6). Does this mean that BL's in various parts of the world do have different etiologies? Alternatively, it is possible that the African BL would be induced by a particular strain of EBV present only within the African tumor belt or that the African environment favors the persistence of EBV genomes in BL cells without any etiological relationship. Finally, one could speculate that the BL's of Africa and most of the so-called BL's outside of Africa would represent diseases of different etiology.

There is evidence in favor of this latter interpretation. We recently observed substantial concentrations of EBV-DNA by reassociation kinetics in a histologically typical BL obtained from a 6-year-old German girl with high antibody titers to EBV antigens (G. W. Bornkamm, H. Stein, K. Lennert, F. Rüggeberg, H. Bartels, and H. zur Hausen, manuscript in preparation). This shows that "typical" BL's occur, although rarely, outside of Africa. Since the number of non-African BL biopsies tested biochemically is still very small, it is reasonable to assume that further studies will reveal more positive cases. Furthermore, it appears that prognosis, response to chemotherapeutic treatment, and mean age differ between African BL's and the American cases.

These data suggest that the biochemical approach contributes to the differential diagnosis of a malignant disease. In view of the pattern emerging from these studies I pro-
posed recently to omit the term "Burkitt's lymphoma" and to replace it by "EBV-associated lymphoma," using the presence of the EBV-genome as a diagnostic criterion for the differentiation of human lymphomas (12). There can be little doubt that the expression of virus-specific antigens within nuclei and membranes of EBV-carrying cells renders them profoundly different from virus-free cells of similar origin. It is felt that the suggested differentiation may lead to consequences as far as prognosis and treatment, as well as research for other etiologies, are concerned.

The specificity of EBV for this type of tumor is underlined by nucleic acid hybridizations performed in our laboratory with more than 50 other B-type lymphomas and more than 40 biopsies from patients with Hodgkin's disease. All of them were found to be free of detectable EBV-DNA, although a substantial proportion of the respective patients revealed high antibody titers against EBV antigens (G. W. Bornkamm, H. Stein, K. Lennert, F. Rüggeberg, and H. zur Hausen, manuscript in preparation).

The only exception observed thus far in our studies is represented by a case of immunoblastic lymphadenopathy. Despite a low antibody level this patient revealed 2 to 3 EBV genome equivalents per cell in lymph nodes removed at autopsy. It remains to be seen whether this rare form of lymphoproliferation shows any consistent association with EBV.

The situation in NPC resembles that of the EBV-associated lymphoma in 1 feature: biochemical studies clearly reveal EBV-DNA within these tumors (13, 14). Moreover, by in situ hybridizations we established the localization of viral DNA within nuclei of epithelial tumor cells (8-10). NPC's collected from different parts of the world reveal the same pattern for the presence of EBV-DNA (10). Biochemical approaches here provided the clue for the consistent involvement of EBV in this human cancer.

It is obvious that many questions concerning the pathogenesis of EBV-induced tumors remain open. Chart 1 offers a tentative scheme emerging from the discussed data which still contains several questions marks. It is speculated that the primary target cell for EBV production is represented by epithelial cells of the lymphoepithelium of the nasopharynx. The release of infectious EBV from these cells leads to infection of bone marrow-derived lymphocytes within tonsils and lymphoepithelium and to their transformation and proliferation. The presence of virus-induced surface antigens in those cells (3) in turn induces proliferation of reactiv thymus-derived cells (atypical lymphocytes), resulting in growth inhibition and destruction of EBV-genome-carrying bone marrow lymphocytes. Survival of transformed cells may occur if they reside within immunologically protected sites. It may also be due to diminished expression of virus-specific surface antigens. Under exceptional circumstances (i.e., immunosuppression) those cells may give rise to EBV-associated lymphomas. If epithelial cells of the nasopharyngeal region allow EBV production, their transformation could be due to defective EBV genomes.

This scheme is very tentative and alternative explanations are possible. It contains, however, predictions that are experimentally accessible. In particular, partial denaturation mapping of EBV-DNA derived from lymphoblastoid lines and NPC's should provide a clue as to whether the virus-specific molecules in the latter tumor contain deletions or originate from a different strain of EBV. Such studies are presently in progress in our laboratory.

![Chart 1. Tentative scheme of EBV-induced pathogenesis.](cancerres.aacrjournals.org)
References


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