Epstein-Barr virus (EBV) DNA in plasma and serum as a tumour marker for EBV-associated malignancies

Y.M. Dennis LO

Department of Chemical Pathology, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, New Territories, Hong Kong Special Administrative Region, China

Abstract

Recently, much interest has been developed on the study of circulating DNA in the plasma of humans. These studies have led to the finding of circulating Epstein-Barr virus (EBV) DNA in the plasma of EBV-associated malignancies, including nasopharyngeal carcinoma, Burkitt's lymphoma and Hodgkin's disease. Clinical data indicate that the detection of plasma/serum EBV DNA holds promise for the diagnosis and monitoring of these EBV-associated cancers. Many biological and clinical questions regarding circulating DNA remain unanswered and would be fruitful areas of research over the next few years.

Keywords: Epstein-Barr virus. Monitoring. Molecular diagnosis.

Introduction

Epstein-Barr virus (EBV) is a common virus which has been associated with many human diseases[1]. Of particular relevance to this review are the wide variety of tumours which are associated with EBV, including nasopharyngeal carcinoma (NPC), Burkitt's lymphoma and Hodgkin's disease[1]. A number of antibody-based blood tests are available for detecting these EBV-associated malignancies[2]. However, many of these tests suffer from a lack of sensitivity or specificity and there is thus a continual search for new tests for EBV-associated malignancies. This review focusses on the potential use of EBV DNA in the plasma and serum as a new tumour marker for this important group of cancers.

Circulating DNA in humans

It has been known for several decades that DNA can be found in the plasma of humans[3-5]. Of particular interest is the demonstration that the concentrations of circulating DNA are increased in patients with cancer[6,7], systemic lupus erythematosus[8] and thromboembolism[9]. Recently, it has been demonstrated that tumour-associated molecular alterations, including oncogene mutations[10-12], microsatellite alterations[13-15], oncogene amplifications[16] and methylation abnormalities[17,18] can be detected in the plasma and serum of cancer patients. These observations therefore suggest that at least a proportion of the circulating DNA in cancer patients is derived from the tumour cells and open up the exciting possibility of using plasma or serum DNA as a new class of tumour markers.

As viral nucleic acids have been detected in a variety of tumour types, it is possible that nucleic acids derived from viruses may also act as tumour markers for viral-associated cancers. This latter possibility is of potential relevance to the detection of EBV-associated malignancies in which EBV DNA has been detected in the tumour tissues[19].

EBV DNA in the plasma or serum of EBV-associated malignancies

One of the first EBV-associated malignancy to be tested for circulating cell-free EBV DNA is NPC, which is a common tumour type in Southern China and Southeast Asia[1]. Thus, Mutirangura et al demonstrated that EBV DNA could be detected in the serum of 31% of NPC patients[20]. Using a sensitive real time quantitative PCR assay for EBV DNA, Lo et al have further enhanced the sensitivity of EBV DNA detection in NPC patients to 96% , while maintaining a high specificity of 93%[21]. Lo et al have also observed a positive correlation between the concentration of circulating EBV DNA and clinical staging, thus indicating that the level of plasma EBV

DNA may be a reflection of the tumour burden in a particular individual[21]. In addition, the levels of circulating EBV DNA have been found to decrease rapidly in those who respond to radiotherapy[21-23]. On the other hand, in individuals who responded poorly or in those with clinical relapse, EBV DNA is persistently detectable and in many cases an increasing trend is observed[24-26]. The latter observation raises the possibility that plasma EBV DNA measurement may be a valuable tool for the early detection of tumour recurrence.

The applicability of circulating EBV DNA analysis to other EBV-associated malignancies has recently been demonstrated by the detection of EBV DNA in the serum of patients with Hodgkin's disease[27,28]. Similar to the studies by Lo et al[21,23,24], Gallagher et al have also utilised a real time quantitative PCR assay in their study[28]. The sensitivity and dynamic range of real time PCR have been reaffirmed by these studies and it is likely that this technique will have an important clinical role to play if and when plasma EBV DNA analysis were to be introduced in routine laboratory of patients with EBV-associated malignancies.

Mechanisms of liberation of EBV DNA

The potential mechanisms responsible for the liberation of cell-free EBV DNA in the plasma of patients with EBV-associated malignancies remain unclear. Mutirangura et al reported an interesting correlation between apoptosis in NPC tissues and the presence of EBV DNA in the serum of NPC patients[20]. Along the same line of reasoning, Lo et al have also reported that there is a transient elevation in the levels of plasma EBV DNA during the first weeks of radiotherapy[23]. These latter observations are consistent with the liberation of EBV DNA following radiation-induced tumoral cell death. Outside the EBV field, plasma DNA has also been suggested by various authors to be related to tumoral and other types of cell death[29-32].

Much work still remains to be done with regard to the elucidation of the molecular nature of the circulating EBV DNA that is found in the plasma of patients with EBV-associated malignancies. Theoretically, circulating EBV DNA could consist of fragments of the EBV genomes, or even intact and potentially infectious virions. The performance of experiments involving DNase which digests 'naked' DNA has suggested that at least a portion of the circulating EBV DNA exists as relatively unprotected DNA fragments[28]. It is evident that further research is necessary to confirm this issue and to possibly quantify the various molecular species of circulating EBV DNA.

Kinetics of EBV DNA clearance

The clearance of circulating DNA has been addressed a number of years ago by investigating the clearance of injected DNA in laboratory animals[33-36]. These early studies have generally found that circulating DNA species are cleared very rapidly from the circulation. However, these early experimental systems are highly artificial, involving the injection of purified DNA, typically isolated from another species, into laboratory animals. The relevance of these studies to the in vivo situation in humans is therefore unclear. Experimental systems with more relevance to humans were developed following the discovery that haemodialysis was associated with the liberation of DNA into the plasma[31,37]. However, it could still be argued that haemodialysis is a non-physiological scenario involving individuals with intrinsic impairment in renal function. The recent discovery of fetal DNA in maternal plasma[38] and the study of the clearance of fetal DNA thus offers the first truly physiological situation for investigating the clearance of DNA of human origin from the circulation[39]. The results from these recent studies confirm previous reports that circulating DNA is indeed cleared from the circulation extremely rapidly, with a half-life of the order of minutes.

The detection of circulating EBV DNA in the plasma of EBV-associated malignancies offers another scenario for the study of DNA clearance from the circulation. Such a study has recently been reported and involved the elucidation of the kinetics of plasma EBV DNA clearance in NPC patients undergoing radiotherapy[23]. This study shows that plasma EBV DNA is cleared from the circulation of NPC patients with a median half-life of 3.8 days (interquartile range: 2.4 to 4.4 days)[23]. Mechanistically, this half-life can be regarded as consisting of two parts: (a) the half-life of EBV DNA liberation into the circulation, most probably as a result of tumour cell death induced by radiation; and (b) the half-life of EBV DNA clearance from the plasma once it has been released. If the rapid kinetics of circulating fetal DNA clearance[39] can be extrapolated to EBV DNA

clearance, then one can argue that most of the observed EBV DNA clearance half-life is taken up by process (a). In other words, the determination of the kinetics of EBV DNA clearance following radiotherapy allows one to estimate the rate of tumour cell death following treatment. With further study, it is envisaged that this may be developed into a method for determining the radiosensitivity of a particular tumour. Apart from NPC, this type of analysis can also be readily applied to other EBV-associated malignancies and other modalities of treatment, such as chemotherapy. It is optimistic that this type of development may allow one to evaluate the in vivo efficacy of new therapeutic modalities for these malignancies.

Functionality of Circulating DNA

An important issue that remains to be addressed is whether DNA in the plasma is transcriptionally active. This question can also be readily applied to circulating fragments of EBV DNA. In this regard it is interesting to note that Holmgren et al observed the uptake of apoptotic bodies of EBV-carrying cell lines by a number of EBV-negative cell types[40]. They reported the surprising observation that the uptake of these apoptotic bodies resulted in the expression of EBV-encoded genes. These results therefore suggested that DNA in apoptotic bodies may be rescued and reused by somatic cells.

Additional evidence that circulating DNA may be functional is provided by Garcia-Olmo et al who demonstrated that plasma from rats carrying cancer cells containing a genomic tag was able to transfer the tag onto cells which did not contain the tag at the beginning of the experiment[41]. These investigators suggested that such transfer of DNA might play a role in tumoral metastasis, a mechanism that they have termed "genometastasis"[41,42]. The potential importance of circulating DNA in the metastatic phenomenon could also be seen in the report by Sugihara et al who observed that the treatment of mice with DNase I reduced the occurrence of liver metastasis in mice transplanted with tumours[43]. It is thus clear that future work studying on the potential functionality of circulating DNA may yield important information of biological and potential clinical significance.

Circulating RNA

Most of the published work on circulating nucleic acids has focussed on circulating DNA, with relatively little attention being paid to the possibility that circulating RNA may also exist. Early work has demonstrated the existence of RNA in the nucleoprotein complex that is released by human lymphocytes and amphibian cells in culture[44]. Similarly, an RNA-proteolipid complex has also been detected in serum samples obtained from patients with a variety of cancer, but not in individuals without malignant disease[45]. Prompted by these early results, our group has recently reported the detection of circulating EBV-associated RNA in the plasma of NPC patients[46]. Kopreski et al have also reported data consistent with ours by detecting tumour-derived tyrosinase mRNA in the serum of patients with malignant melanoma[47]. Taken together, these data strongly support the existence of circulating tumour-derived RNA in the cell-free fraction of blood in cancer patients. Further work would be required to establish quantitative parameters governing circulating RNA, and to correlate the relative levels of plasma RNA and DNA. Theoretically, the ability to detect circulating tumour-derived mRNA would provide a non-invasive handle on the transcriptional activity of a particular tumour, both at presentation and during treatment.

Conclusions

In conclusion the recent scientific interest in circulating nucleic acids has provided many powerful tools in the diagnosis and monitoring of EBV-associated malignancies. Early data have indicated that plasma DNA-based markers might be some of the most accurate and clinically relevant markers for these malignancies. Apart from EBV-associated cancers, these findings might also be extrapolated to other biological and clinical aspects of circulating nucleic acids. As many questions remain unanswered, it is hoped that studies over the next few years would further enhance our understanding of this emerging field.

References

1. Rickinson AB, Kieff E. Epstein-Barr Virus. In: Fields Virology. Fields BN, Knipe DM, Howley PM, ed. Third Edition ed. Philadelphia: Lippincott-Raven Publishers, 1996: 2397-2446.
2. Tam JS. Epstein-Barr virus serological markers. In: Nasopharyngeal Carcinoma. van Hasselt CA, Gibb AG, ed. Second ed. Hong Kong: The Chinese University Press, 1999: 161-176.
3. Tan EM, Schur PH, Carr RI, Kunkel HG. Deoxyribonucleic acid (DNA) and antibodies to DNA in the serum of patients with systemic lupus erythematosus. J Clin Invest 1966; 45: 1732-1740.
4. Davis GLJ, Davis JSI. Detecting of circulating DNA by counterimmunoelectrophoresis (CIE). Arthritis Rheum 1973; 16: 52-58.
5. Dennin RH. DNA of free and complexed origin in human plasma: concentration and length distribution. Klin Wochenschr 1979; 57: 451-456.
6. Leon SA, Shapiro B, Sklaroff DM, Yaros MJ. Free DNA in the serum of cancer patients and the effect of therapy. Cancer Res 1977; 37: 646-650.
7. Shapiro B, Chakrabarty M, Cohn EM, Leon SA. Determination of circulating DNA levels in patients with benign or malignant gastrointestinal disease. Cancer 1983; 51: 2116-2120.
8. Steinman CR. Circulating DNA in systemic lupus erythematosus: isolation and characterisation. J Clin Invest 1984; 73: 832-841.
9. Vargo JS, Becker DM, Philbrick JT, et al. Plasma DNA: A simple, rapid test for aiding the diagnosis of pulmonary embolism. Chest 1990; 97: 63-68.
10. Vasioukhin V, Anker P, Maurice P, et al. Point mutations of the N-ras gene in the blood plasma DNA of patients with myelodysplastic syndrome or acute myelogenous leukaemia. Br J Haematol 1994; 86: 774-779.
11. Sorenson GD, Pribish DM, Valone FH, et al. Soluble normal and mutated DNA sequences from single-copy genes in human blood. Cancer Epidemiol Biomarkers Prev 1994; 3: 67-71.
12. Anker P, Lefort F, Vasioukhin V, et al. K-ras mutations are found in DNA extracted from the plasma of patients with colorectal cancer. Gastroenterology 1997; 112: 1114-1120.
13. Chen XQ, Stroun M, Magnenat J-L, et al. Microsatellite alterations in plasma DNA of small cell lung cancer patients. Nat Med 1996; 2: 1033-1035.
14. Nawroz H, Koch W, Anker P, et al. Microsatellite alterations in serum DNA of head and neck cancer patients. Nat Med 1996; 2: 1035-1037.
15. Chen XQ, Bonnefoi H, Diebold-Berger S, et al. Detecting tumor-related alterations in plasma or serum DNA of patients diagnosed with breast cancer. Clin Cancer Res 1999; 5: 2297-2303.
16. Chiang P-W, Beer DG, Wei W-L, et al. Detection of erbB-2 amplifications in tumors and sera from esophageal carcinoma patients. Clin Cancer Res 1999; 5: 1381-1386.
17. Wong IHN, Lo YMD, Zhang J, et al. Detection of aberrant p16 methylation in the plasma and serum of liver cancer patients. Cancer Res 1999; 59: 71-73.
18. Esteller M, Sanchez-Cespedes M, Rosell R, et al. Detection of aberrant promoter hypermethylation of tumor suppressor genes in serum DNA from non-small cell lung cancer patients. Cancer Res 1999; 59: 67-70.
19. Huang DP, Lo KW. Aetiological factors and pathogenesis. In: Nasopharyngeal Carcinoma. van Hasselt CA, Gibb AG, ed. Second ed. Hong Kong: The Chinese University Press, 1999: 31-60.
20. Mutirangura A, Pornthanakasem W, Theamboonlers A, et al. Epstein-Barr viral DNA in serum of patients with nasopharyngeal carcinoma. Clin Cancer Res 1998; 4: 665-669.
21. Lo YMD, Chan LYS, Lo KW, et al. Quantitative analysis of cell-free Epstein-Barr virus DNA in plasma of patients with nasopharyngeal carcinoma. Cancer Res 1999; 59: 1188-1191.
22. Shotelersuk K, Khorprasert C, Sakdikul S, et al. Epstein-Barr virus DNA in serum/plasma as a tumor marker for nasopharyngeal cancer. Clin Cancer Res 2000; 6: 1046-1051.
23. Lo YMD, Leung SF, Chan LYS, et al. Kinetics of plasma Epstein-Barr virus DNA during radiation therapy for nasopharyngeal carcinoma. Cancer Res 2000; 60: 2351-2355.
24. Lo YMD, Chan LYS, Chan ATC, et al. Quantitative and temporal correlation between circulating cell-free Epstein-Barr virus DNA and tumor recurrence in nasopharyngeal carcinoma. Cancer Res 1999; 59: 5452-5455.
25. Yip TTC, Cho CS, Lau WH, et al. PCR detection of DNA encoding Epstein-Barr virus latent membrane protein-1 in cell free sera of nasopharyngeal carcinoma patients with distant or loco-regional relapses but not in those in complete remission. Proc Amer Assoc Cancer Res 1998; 39: abstract 1559.
26. Yip TTC, Cho CS, Lau WH, et al. Serum

amplification of Epstein-Barr virus genes is a novel and useful predictor for relapse of nasopharyngeal carcinoma. Proc Amer Assoc Cancer Res 1999; 40: abstract 4809.
27. Drouet E, Brousset P, Fares F, et al. High Epstein-Barr virus serum load and elevated titers of anti-ZEBRA antibodies in patients with EBV-harboring tumor cells of Hodgkin's disease. J Med Virol 1999; 57: 383-389.
28. Gallagher A, Armstrong AA, MacKenzie J, et al. Detection of Epstein-Barr virus (EBV) genomes in the serum of patients with EBV-associated Hodgkin's disease. Int J Cancer 1999; 84: 442-448.
29. Fournie GJ, Martres F, Pourrat JP, et al. Plasma DNA as cell death marker in elderly patients. Gerontology 1993; 39: 215-221.
30. Fournie GJ, Courtin JP, Laval F, et al. Plasma DNA as a marker of cancerous cell death. Investigations in patients suffering from lung cancer and in nude mice bearing human tumours. Cancer Letts 1995; 91: 221-227.
31. Fournie GJ, Lule J, Dueymes J-M, et al. Plasma DNA in patients undergoing hemodialysis or hemofiltration: cytolysis in artificial kidney is responsible for the release of DNA in circulation. Am J Nephrol 1989; 9: 384-391.
32. Lo YMD, Rainer TH, Chan LYS, et al. Plasma DNA as a prognostic marker in trauma patients. Clin Chem 2000; 46: 319-323.
33. Tsumita T, Iwanaga M. Fate of injected deoxyribonucleic acid in mice. Nature 1963; 198: 1088-1089.
34. Chused TM, Steinberg AD, Talal N. The clearance and localization of nucleic acids by New Zealand and normal mice. Clin Exp Immunol 1972; 12: 465-476.
35. Emlen W, Mannik M. Kinetics and mechanisms for removal of circulating single-stranded DNA in mice. J Exp Med 1978; 147: 684-699.
36. Emlen W, Mannik M. Effect of DNA size and strandedness on the in vivo clearance and organ localization of DNA. Clin Exp Immunol 1984; 56: 185-192.
37. Steinman CR, Ackad A. Appearance of circulating DNA during hemodialysis. Am J Med 1977; 62: 693-697.
38. Lo YMD, Corbetta N, Chamberlain PF, et al. Presence of fetal DNA in maternal plasma and serum. Lancet 1997; 350: 485-487.
39. Lo YMD, Zhang J, Leung TN, et al. Rapid clearance of fetal DNA from maternal plasma. Am J Hum Genet 1999; 64: 218-224.
40. Holmgren L, Szeles A, Rajnavolgyi E, et al. Horizontal transfer of DNA by the uptake of apoptotic bodies. Blood 1999; 93: 3956-3963.
41. Garcia-Olmo D, Garcia-Olmo DC, Ontanon J, et al. Tumor DNA circulating in the plasma might play a role in metastasis. The hypothesis of the genometastasis. Histol Histopathol 1999; 14: 1159-1164.
42. Garcia-Olmo D, Garcia-Olmo DC, Ontanon J, Martinez E. Horizontal transfer of DNA and the "genometastasis hypothesis". Blood 2000; 95: 724-725.
43. Sugihara S, Yamamoto T, Tanaka H, et al. Deoxyribonuclease treatment prevents blood-borne liver metastasis of cutaneously transplanted tumour cells in mice. Br J Cancer 1993; 67: 66-70.
44. Stroun M, Anker P, Beljanski M, et al. Presence of RNA in the nucleoprotein complex spontaneously released by human lymphocytes and frog auricles in culture. Cancer Res 1978; 38: 3546-3554.
45. Wieczorek AJ, Rhyner C, Block LH. Isolation and characterization of an RNA-proteolipid complex associated with the malignant state in humans. Proc Natl Acad Sci USA 1985; 82: 3455-3459.
46. Lo KW, Lo YMD, Leung SF, et al. Analysis of cell-free Epstein-Barr virus-associated RNA in the plasma of patients with nasopharyngeal carcinoma. Clin Chem 1999; 45: 1292-1294.
47. Kopreski MS, Benko FA, Kwak LW, Gocke CD. Detection of tumor messenger RNA in the serum of patients with malignant melanoma. Clin Cancer Res 1999; 5: 1961-1965.

Correspondence to: Professor Y.M. Dennis Lo
email: loym@cuhk.edu.hk