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ORIGINAL ARTICLE

Ethel-Michele de Villiers · Rainer Schmidt
Hajo Delius · Harald zur Hausen

Heterogeneity of TT virus related sequences isolated from human tumour biopsy specimens

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Abstract TT viruses have recently been reported in serum samples from varying percentages of human blood donors and in patients with chronic liver disease. However, no association with human pathology has yet been reported. In a pilot study we analysed 162 biopsy specimens from various human cancers and from colon polyps for the presence of TT virus related sequences by polymerase chain reaction using three sets of nested primers for TT virus detection. All gels were subjected to Southern blot hybridisation, and DNA from hybridising bands was cloned and sequenced. A total of 54.3% of tumour specimens contained identifiable TT virus related sequences. Specimens from hypopharynx, larynx, endometrial, ovarian and bladder cancers were 14–35% positive and gastrointestinal cancers (oesophagus, stomach, colon, rectum) and colon polyps 57–100% positive. Lung cancers (68.4%), mammary cancers (50%), multiple myelomas (85.7%) and human leukaemias (53.3%) also revealed a high prevalence of TT virus related sequences. Since normal control tissues were not available for the tumour biopsy specimens tested, these data do not permit conclusions concerning a possible causal relationship between the virus infections and carcinogenesis. Another aspect, however, deserves attention: the heterogeneity of TT virus clones obtained from the specimens tested here was striking: 66 novel sequences, probably belonging to new types were identified. Only 16 clones corresponded by more than 97% of their sequences to established prototypes. Even individual tumours commonly contained sequences substantially diverging in nucleic acid composition. Up to five different types were identified within an individual tumour. The high variability of these virus types suggests that additional primer combinations within the highly conserved region of the genome would detect a still higher rate of positive tumours.

Keywords TT virus · Viral infection of human tumours · Heterogeneity of TT virus isolates

Abbreviations *TTV:* TT virus

ETHEL-MICHELE DE VILLIERS received a D.Sc. degree from the University of Pretoria, South Africa. She is presently Professor and Head of the Division for Tumor Virus Characterization at the Deutsches Krebsforschungszentrum in Heidelberg. Her main research interest is searching for viruses that may be associated with the aetiology of malignant tumours.

E.-M. de Villiers (✉) · R. Schmidt · H. Delius · H. zur Hausen
Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany
e-mail: e.devilliers@dkfz-heidelberg.de
Fax: +49-6221-424822

Introduction

It is presently estimated that 15% of the global cancer burden is due to virus infections [1, 2]. Particularly papillomavirus infections in anogenital cancers and hepatitis B and C viruses in hepatocellular carcinomas contribute to this figure. During the past 10 and increasingly during the past 3 years a number of additional viruses have been characterized that are linked to human cancers or stimulate host cell proliferation [2]. These are hepatitis C, human herpesvirus type 8, and novel types of "cutaneous" papillomavirus types [3]. This leads to the speculation that additional infectious agents with a possible relationship to human cancers may exist.

We set up a screening program for the analysis and characterisation of viruses in human tumour biopsy specimens. In a previous publication we reported negative findings by screening various cancer specimens for members of the polyoma virus family by using consen-

sus primers and PCR technology [4]. These studies were now extended to the recently discovered TT virus (TTV) family. This family was initially discovered by Nishizawa and colleagues [5] by analysing sera from post-transfusion non-B, non-C hepatitis patients by representational difference analysis. Based on the sedimentation properties of the first isolate in sucrose gradient centrifugation and on DNase I sensitivity of the nucleic acid, Nishizawa et al. concluded that the cloned nucleic acid originated from a DNA virus containing single stranded DNA. The designation TTV originated from the initials of the patient from whom the DNA had been isolated.

During the past 3 years a number of studies have confirmed the original observation by Nishizawa and colleagues. TTV-related virus isolates obtained from various parts of the world revealed a remarkable type and subtype heterogeneity. At present a large number of types and subtypes have been described, some of them varying substantially in their nucleic acid composition [6, 7, 8, 9, 10, 11]. Their viral genomes contain three to five open reading frames and a long noncoding region [12]. Various isolates ranging between 2300 and 3800 nucleotides in genome length have been reported.

The reports published thus far failed to show any pathogenicity of members of this virus group [13], now tentatively designated as the family Circinoviridae [14]. Many attempts to link this infection to pathological liver conditions have been unsuccessful. The studies conducted so far suggest a long-term persistence of these viruses in the peripheral blood and major geographic variations in TTV prevalence. One surprising aspect of TTV infection is the high prevalence of up to 95% in normal blood donors [15] and the variation in geographic distribution of types [16]. Some evidence exists for increasing TTV infections with age, blood transfusions, immunosuppression and chronic liver disease [17, 18, 19, 20]. To our knowledge, with the exception of hepatocellular carcinomas [21, 22, 23] and haematopoietic disorders [24], this virus family has not been systematically investigated for a possible involvement in human tumours.

In this report we describe a relatively high prevalence of TTV-related DNA sequences in specific human cancers, most notably in cancers of the gastrointestinal tract, lung and breast but also in a number of multiple myelomas. In addition, a surprising degree of heterogeneity is reported for individual isolates from specific tumours.

Materials and methods

Samples and DNA extraction

Tumour samples were collected over a period of 25 years from various hospitals and geographical areas. The samples analysed in this study included carcinomas of the larynx (*n*=20), hypopharynx (*n*=13), lung (*n*=19), breast (*n*=10), oesophagus (*n*=14), stomach (*n*=9), colon (*n*=13), rectum (*n*=7), bladder (*n*=12), ovaries (*n*=9) and endometrium *n*= (7) as well as multiple myelomas (*n*=7), leukaemias (*n*=15) and colon polyps (*n*=7). All biopsy specimens were frozen and stored at –70°C until use. DNA extraction was performed as described previously [25].

PCR analysis and cloning

Total cellular DNA (100 ng/sample) was amplified by PCR. Primers (sets A, B and C) used for the initial amplification and subsequent nested amplification and the amplification conditions are described by Leary et al. [26]. The primer set A amplified the region between nucleotides 94–606 of the TTV TA278 (GenBank accession no. AB008394), primer set B between nucleotides 3087 and 3392, and primer set C between nucleotides 3293 and 3641. Aliquots of the nested products were separated by electrophoresis (2% agarose gels) and blotted onto nylon membranes. Hybridisation was performed in 50% formamide, 5× SCC, 1% SDS and Denhardt's solution at 42°C, using the P/1C1 complete genome (accession no. AF298585, kindly provided by Dr. Schreier) as radiolabelled probe. Washing was performed at 68°C in the presence of 2× SCC and 0.1% SDS. PCR products corresponding to the hybridisation signals were eluted and cloned into the pMOSBlue vector (Invitrogen). Up to six inserts of each PCR product were sequenced using the Sequenase 2.0 DNA Sequencing Kit (USB, Cleveland). The final sequences were determined on both strands on an ABI Model Sequencer using the Big Dye Terminator chemistry (Perkin Elmer Applied Biosystems Division).

Sequence analysis

Sequences were compared to the available TTV sequences in the EMBL and GenBank databanks using the Husar software package (Deutsches Krebsforschungszentrum). Sequences of putative new TTV types have been deposited in the EMBL Nucleotide Sequence Database and the accession numbers are AJ315402 to AJ315450.

Results

A total of 162 tumour biopsy specimens, including DNA from 22 haematopoietic malignancies, was tested for the presence of TTV-related sequences by using the three different primer sets outlined before. A specimen was regarded as positive if a hybridisation signal was observed with the amplicon resulting from one of the primer sets. Nonspecific amplification products were observed for all three primer sets after gel electrophoresis and the amplification products of only eight specimens showed a positive hybridisation signal with two primer sets and two specimens with all three primer sets. Primer set C resulted in a positive result in 86 specimens, primer set B in 9 positive samples and primer set A in 6 positive specimens. In preparations with prominent bands or those identified within a complex banding pattern after Southern blot hybridisation (Fig. 1), the respective regions were excised after gel electrophoresis of the PCR products, the DNA was cloned and at least six clones subsequently sequenced. Table 1 provides the data obtained after Southern blot hybridisation of the gels. A total of 54.3% of tumours revealed positive hybridisation signals.

Inspection of individual tumour groups revealed remarkable differences in the number of positive signals.

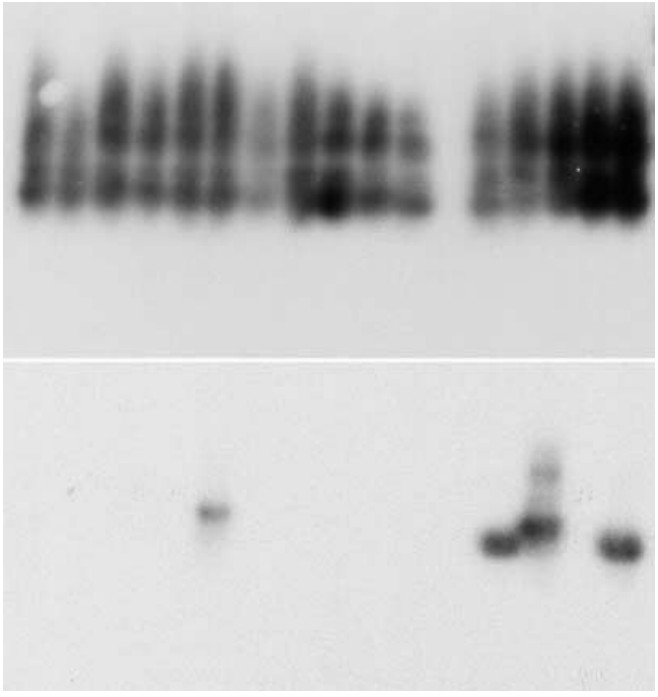

Fig. 1 Southern blot hybridisation of DNA from oesophageal (*top*) and breast cancer (*bottom*) biopsy specimens amplified with TT virus consensus primers and hybridised with a radiolabelled probe of the complete genome P/1C1

Hypopharynx, larynx, endometrial, ovarian and bladder cancers ranged between 14% and 35% of positive signals while tumours of the oesophagus and the gastrointestinal tract revealed between 57% and 100% of positive data. In addition, lung, breast and haematopoietic malignancies were 50–86% positive. Comparing of different tumour groups showed that the hybridisation signals differed substantially among the tumour types. For example, breast, larynx and colon cancer PCR products revealed comparatively sharp bands, while particularly those from oesophageal cancers exhibited a remarkably heterogeneous pattern. Hybridisation signals of PCR products from other tumour types showed a mixed pattern, some with sharp bands others with signals varying considerably in size. At present we cannot exclude that this pattern resulted from a PCR artefact (e.g., recombination of one or more of the amplified fragments), and further studies are required for clarification.

Hybridising DNA was cloned and sequenced only from a number of positive tumours. Of 123 clones containing TTV-related sequences 49 clones obtained from solid tumours contained novel sequences differing from established prototype sequences available in the data bank by more than 3% (Table 2). The isolates listed were obtained after amplification with primer set C, except for isolates 197a-33, 202-24, and 135-1, which were obtained by amplification with primer set A (Fig. 2a). All positive amplicons resulting from primer set B harboured the same TTV-related sequences as the parallel amplicons (from the same tumours) resulting from the primer set C (Fig. 2b). The closest homologies of the new isolates ranged through all the genotypic groups of TTV-related viruses: groups 1, 2 and 3 of TTVs, SANBAN, PMV, SENV and TLMV groups.

From individual tumour biopsy specimens up to five different TTV-related sequences were obtained, underlining the heterogeneity of these agents within the same type of lesion. Table 2 lists the TTV-related novel individual sequences and their degree of relatedness to established TTV and SEN virus sequences available in the data bases. Figure 2 presents neighbour-joining phylogenetic trees constructed on the sequences overlapping the same region of the TTV genome. Figure 2a presents the isolates obtained with the primer set A and Fig. 2b those obtained with primer set C.

Table 1 TTV-related sequences in human tumours

Tumour type	Positive specimens	Negative specimens	Percentage positive
Oesophagus carcinoma	14	0	100.0
Stomach carcinoma	7	2	77.8
Colon polyp	4	3	57.1
Colon carcinoma	9	4	69.2
Rectum carcinoma	4	3	57.1
Gastrointestinal tumours, total	**38**	**12**	**76.0**
Hypopharynx carcinoma	4	9	30.8
Larynx carcinoma	7	13	35.0
Lung carcinoma	13	6	68.4
Mammary carcinoma	5	5	60.0
Endometrial carcinoma	1	6	14.3
Ovarial carcinoma	3	6	33.3
Bladder carcinoma	3	9	25.0
Acute and myeloid leukaemia	8	7	53.3
Multiple myeloma	6	1	85.7
Total number of tumours tested	88	74	54.3

Table 2 Heterogeneity of TTV-related sequences in human tumours

Tumour type	Total number of TTV-related clones sequenced	Novel genotypes	Percentage homology to closest prototype in data base as of 1 October 2000
Oesophagus carcinoma	29 (from 5 tumours)	192a-2[a]	a: 95% ab028308 b: 95% ab028308
		192a-4	91% ab028308
		192a-6[a]	a: 92% ab028308 b: 91% ab028308
		194a-5	93% ab028303
		194a-6[a]	a: 95% ab028306 b: 91% ab028308
		194b-5	90% ab028306
		194b-6	91% af247138
		197a-33	96% ab024360
		202–24	97% ab024360
Colon polyps	12 (from 3 tumours)	206–17	81% af122919
		208–9	91% ab028301
		208–18[a]	a: 91% ab028301 b: 79% af122914
Colon carcinoma	12 (from 4 tumours)	120a-1	93% ab028303
		120a-5[d]	96% ab028303
		125b-3[d]	95% ab028303
		125b-4	92% af247138
		125b-5	96% ab028303
		127–3	82% ab028299
		129–1[b]	a: 94% ab028307
Rectum carcinoma	11 (from 3 tumours)	147a-1[d]	96% ab028303
		147b-2	85% af247138
		147b-3	95% ab028303
		150b-5	91% af122917
Breast carcinoma	14 (from 4 tumours)	81–7[b,d]	a: 94% ab028303
		87–7	83% af122913
		88–10	93% ab028303
		102c-2	96% ab028303
Endometrial carcinoma	1	210a-1	86% af261761
Ovarian carcinoma	14 (from 2 tumours)	152a-2	95% ax025677
		154–2	94% ab028303
		154–6	97% ab028303
Bladder carcinoma	9 (from 2 tumours)	135–1	95% af122920
		141a-4[b,d]	a: 94% ab028303
		141b-3	79% af122919
		141c-5[d]	93% ab028303
Laryngeal carcinoma	9 (from 3 tumours)	7–1	85% af247138
		7–2	94% ax025677
		15a-2	91% ab028303
		16–12	89% af122914
Lung carcinoma	12 (from 4 tumours)	32–1	84% af122914
		32–4	84% ab028669
		32–6	95% ab028304
		39–2	94% ab028304
		40–3[a]	a: 94% af122914 b: 95% af122919
		45a-6	94% ab028303
		45b-1	96% ab029307
		45b-4[c]	88% af122914
		45b-5	86% af122917
		45b-6	94% ab028303

[a] Clone consists of two fragments, each a TTV-related sequence
[b] Clone consists of two fragments, one a TTV-related sequence and the other with homology to cellular sequences
[c] Harbours a deletion within the TTV-related sequence
[d] Clones with sequence homology (>97%) to one or more clones isolated from other tumours

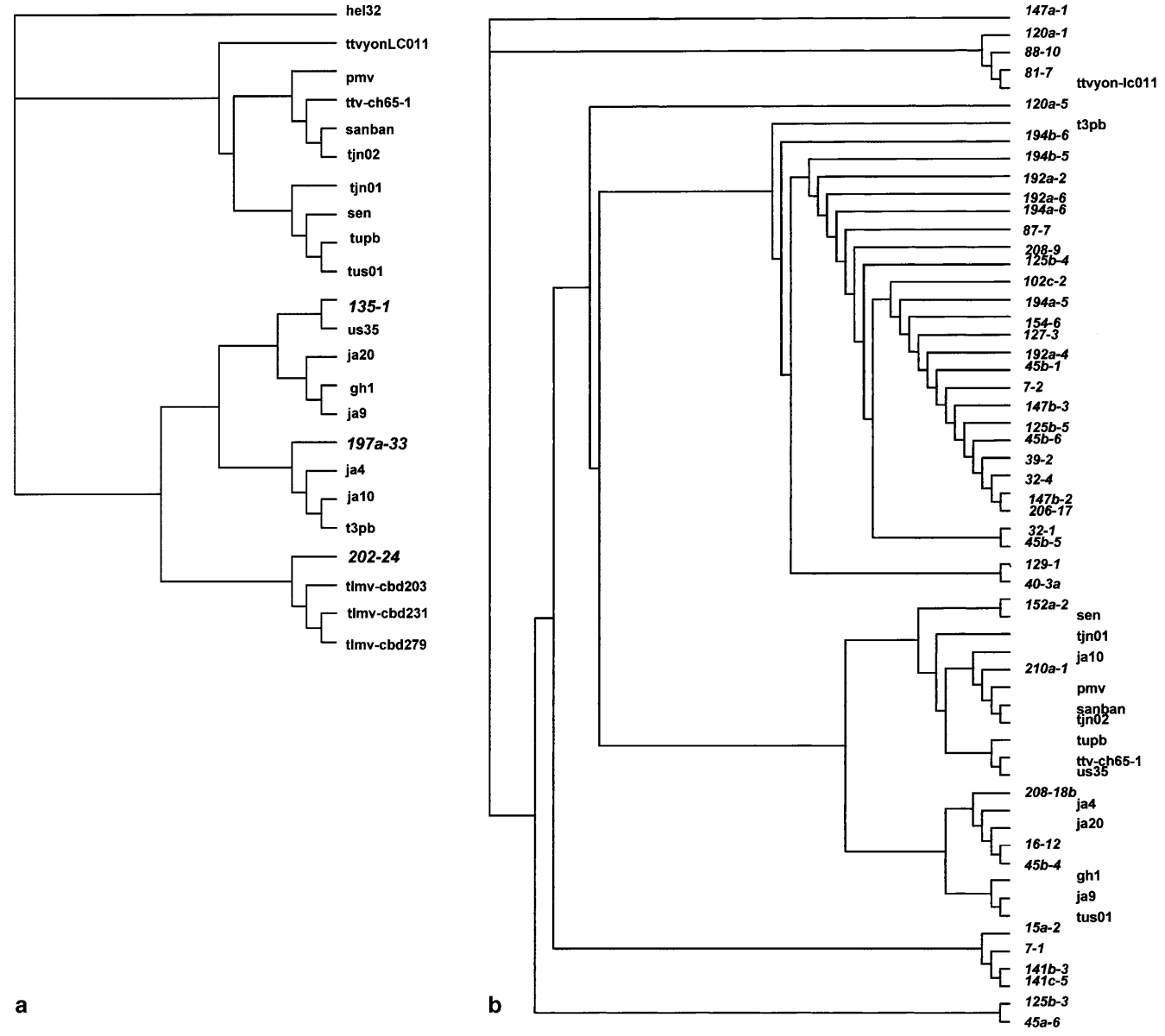

Fig. 2a, b Phylogenetic tree constructed by the neighbour-joining method. TTV-related isolates obtained by amplification with primer set A (**a**) and isolates obtained by amplification with primer set C (**b**). These isolates were compared to 17 TTV genomes (TUPB, accession number AF247137; JA9, AF122915; T3PB, AF247138; JA10, AF122919; JA20, AF122914; JA4, AF122917; GH1, AF122913; US35, AF122920; PMV, AF261761; SEN, AX025677; SANBAN, AB025946; TUS01, AB017613; TJN01, AB028668; TJN02, ab028669; TTV-CH65-1, AB037926; TTVyon-LC011, AB038622; HEL32/6a, AY034068) and three TLMV isolates (TLMV-CBD231, AB026930; TLMV-CBD279, AB026931; TLMV-CBD203, AB026929)

Discussion

Within the 3 years since their initial discovery a large number of TTV genotypes have been recorded, revealing a puzzling heterogeneity of this virus family. The biological reasons for this phenomenon remain obscure, although obviously a high rate of recombinatory events occurs in the course of TTV infections [27]. The long-term persistence of TTV genotypes in the peripheral blood of apparently healthy individuals [15] suggests that these viruses somehow escape immunological surveillance, although detailed studies on immunological responses are still not available.

The present study underlines the heterogeneity of the TTV group and the frequent occurrence of mixed infections [7, 14]. The isolates of TTV-related sequences from human cancer biopsy specimens vary remarkably in sequence variation and heterogeneity. Only 16 of more than 100 sequenced clones with a TTV-characteristic structure corresponded to established prototypes, and the closest homologies to known isolates cover the whole spectrum of TT-related viruses. It is tempting to speculate that the respective total genome would reveal even a higher degree of sequence variation.

It remains an open question whether the cloned DNA of TTV-related sequences originated from the cancer

cells of the respective biopsy specimens or from contaminating blood cells or plasma constituents. TTV persists for prolonged periods of time within the peripheral blood and may thus be transported to tumour sites without infecting the cancer cells [15, 18]. Some findings, however, do suggest that specific cancer types should harbour the viral DNA at least temporarily within the tumour tissue: the high degree of genetic diversity observed between and within individual tumour types seems to exceed the reported diversity of TT viral sequences detected in peripheral blood samples. This is particularly pronounced by the high rate of diverging clones from oesophageal, lung and rectum cancers. In addition, the observed differences in the percentage of various tumour groups positive for TTV-related sequences suggest that the tumour type influences the rate of positivity. A more direct proof should result from in situ hybridisation data which at least for liver cells demonstrate viral DNA persistence within the parenchymal tissue [28]. Since normal control tissues were not available for the tumour biopsy specimens tested, these data do not permit conclusions concerning a possible causal relationship between the virus infections and carcinogenesis.

The remarkable differences noted in this study in the prevalence of TTV-related sequences between various tumour groups deserve some discussion. Although the numbers tested are still too small to permit firm conclusions, a trend towards higher positive rates is observed for tumours of the oesophagus and the gastrointestinal tract. Excretion of TTV via saliva, bile and faeces has been reported [16, 29, 30]; thus sites of the gastrointestinal tract may be preferentially infected, or alternatively tumours may be contaminated with virus produced at other locations within the digestive tract. On the other hand, hybridisation signals obtained from cancers of the oesophagus (Fig. 1) were particularly heterogeneous in size, suggesting to remarkable size heterogeneity of the respective sequences which corresponds also to substantial differences in individual clones isolated from these tumours. Other tumour types (e.g., breast, larynx and colon cancers) revealed more homogeneous hybridisation patterns. The elevated rate of TTV-related DNA sequences in lung tumours deserves further studies.

The primer sets used in this study differed substantially in detecting TTV-related sequences in the various biopsy specimens and frequently failed to result in concordant data. This is obviously caused by the substantial sequence variations observed within the individual clones. It is therefore likely that the use of additional primer combinations will result in an even higher rate of TTV detection within malignant but possibly also within non-malignant tissues [31].

The high degree of sequence variation and the absence of a specific pattern of TTV-related sequences within individual tumour groups argue against a possible involvement of these viruses in the development of the TTV sequence-positive tumour types. The existence of viral quasispecies of TTVes has been reported [32]. In our series (Fig. 2) such quasispecies may be present in some of the tumour samples (e.g., oesophageal carcinoma samples 192 and 194), although the sequence variation between TTV-related isolates in other tumours (e.g., lung tumour sample 45) is more diverse. On the other hand, however, the high degree of variability may occasionally result in accidental recombination of types [27] which eventually become oncogenic and therefore differ among individual tumours. Further studies on growth-promoting potential of TTV genomes persisting in malignant tissue should clarify this question.

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References

1. Hausen H zur (1986) Intracellular surveillance of persisting viral infections: human genital cancer resulting from failing cellular control of papillomavirus gene expression. Lancet II:489–491
2. Hausen H zur (1999) Viruses in human cancers. Eur J Cancer 35:1878–1885
3. Astori G, Lavergne D, Benton C, Höckmayr B, Egawa K, Garbe C, de Villiers E-M (1998) Human papillomaviruses are commonly found in normal skin of immunocompetent hosts. J Invest Dermatol 110:752–755
4. Völter C, zur Hausen H, Alber, D, de Villiers E-M (1997) Screening human tumour samples with a broad spectrum PCR method for the detection of polyomaviruses. Virology 237:389–396
5. Nishizawa T, Okamoto H, Konishi K, Yoshizawa H, Miyakawa Y, Mayumi M (1997) DNA virus (TTV) associated with elevated transaminase levels in posttransfusion hepatitis of unknown etiology. Biochem Biophys Res Commun 41:92–96
6. Ukita M, Okamoto H, Nishizawa T, Tawara A, Takahashi M, Iizuka H, Miyakawa Y, Mayumi M (2000) The entire nucleotide sequences of two distinct TT virus (TTV) isolates (TJN01 and TJN02) remotely related to the original TTV isolates. Arch Virol 145:1543–1559
7. Okamoto H, Takahashi M, Nishizawa T, Ukita M, Fukuda M, Tsuda F, Miyakawa Y, Mayumi M (1999) Marked genomic heterogeneity and frequent mixed infections of TT virus demonstrated by PCR with primers from coding and non-coding regions. Virology 259:428–436
8. Hallett RL, Clewley JP, Bobet F, McKiernan PJ, Teo CG (2000) Characterization of a highly divergent TT virus genome. J Gen Virol 81:2273–2279
9. Takahashi K, Iwasa Y, Hijikata M, Mishiro S (2000) Identification of a new human DNA virus (TTV-like mini virus, TLMV) intermediately related to TT virus and chicken anemia virus. Arch Virol 145:979–993
10. Biagini P, Attoui H, Gallain P, Touinssi M, Cantaloube J-F, de Micco P, de Lamballerie X (2000) Complete sequences of two highly divergent european isolates of TT virus. Biochem Biophys Res Commun 271:837–841
11. Tanaka Y, Primi D, Wang RY, Umemura T, Yeo AE, Mizokami M, Alter HJ, Shih JW (2001) Genomic and molecular evolutionary analysis of a newly identified infectious agent (SEN virus) and its relationship to the TT virus family. J Infect Dis 183:359–367

12. Kamahora T, Hino S, Miyata H (2000) Three spliced mRNAs of TT virus transcribed from a plasmid containing the entire genome in COS1 cells. J Virol 74:9980–9986
13. Bowden S (2001) New hepatitis viruses: contenders and pretenders. J Gastroenterol Hepatol 16:124–131
14. Mushawar IK, Erker JC, Muerhoff AS, Leary TP, Simons JN, Birkenmeyer LG, Chalmers ML, Pilot-Matias TJ, Desai SM (1999) Molecular and biophysical characterization of TT virus: evidence for a new virus family infecting humans. Proc Natl Acad Sci USA 96:3177–3182
15. Okamoto H, Takahashi M, Kato N, Fukuda M, Tawara A, Fukuda S, Tanaka T, Miyakawa Y, Mayumi M (2000) Sequestration of TT virus of restricted genotypes in peripheral blood mononuclear cells. J Virol 74:10236–10239
16. Gallian P, Biagini P, Zhong S, Touinssi M, Yeo W, Cantaloube JF, Attoui H, de Micco P, Johnson PJ, de Lamballerie X (2000) TT virus: a study of molecular epidemiology and transmission of genotypes 1, 2 and 3. J Clin Virol 17:43–49
17. Forns X, Hegerich P, Darnell A, Emerson SU, Purcell RH, Bukh J (1999) High prevalence of TT virus (TTV) infection in patients on maintenance hemodialysis: frequent mixed infections with different genotypes and lack of evidence of associated liver disease. J Med Virol 59:313–317
18. Hsieh SY, Wu YH, Ho YP, Tsao KC, Yeh CT, Liaw YF (1999) High prevalence of TT virus infection in healthy children and adults and in patients with liver disease in Taiwan. J Clin Microbiol 37:1829–1831
19. Saback FL, Gomes SA, de Paula VS, da Silva RR, Lewis-Ximenez LL, Niel C (1999) Age-specific prevalence and transmission of TT virus. J Med Virol 59:318–322
20. Christensen JK, Eugen-Olsen J, Sørensen M, Ullum H, Gjedde SB, Pedersen BK, Nielsen JO, Krogsgaard K (2000) Prevalence and prognostic significance of infection with TT virus in patients infected with human immunodeficiency virus. J Infect Dis 181:1796–1799
21. Yotsuyanagi H, Shintani Y, Moriya K, Fujie H, Tsutsumi T, Kato T, Nishioka K, Takayama T, Makuuchi M, Iino S, Kimura S, Koike K (2000) Virologic analysis of non-B, non-C hepatocellular carcinoma in Japan: frequent involvement of hepatitis B virus. J Infect Dis 181:1920–1928
22. Kim SR, Hayashi Y, Kudo M, Imoto S, Ando K, Shintani S, Koterazawa T, Kim KI, Taniguchi M (2000) TTV positivity and transfusion history in non-B, non-C hepatocellular carcinoma compared with HBV- and HCV-positive cases. Intervirology 43:13–15
23. Pineau P, Meddeb M, Raselli R, Qin LX, Terris B, Tang ZY, Tiollais P, Mazzaferro V, Dejean A (2000) Effects of TT virus infection on hepatocellular carcinoma development: results of a Euro-Asian study. J Infect Dis 181:1138–1142
24. Tanaka Y, Mizokami M, Orito E, Ohno T, Nakano T, Kato T, Iida S, Ueda R (2000) Lack of integrated TT virus (TTV) genomes into cellular DNA in infected human hematopoietic cells. Leuk Lymphoma 38:411–417
25. Lavergne D, de Villiers, E-M (1999) Papillomavirus in esophageal papillomas and carcinomas. Int J Cancer 80:681–684
26. Leary TP, Erker JC, Chalmers ML, Desai SM, Mushahwar IK (1999) Improved detection systems for TT virus reveal high prevalence in humans, non-human primates and farm animals. J Gen Virol 80:2115–2120
27. Worobey M (2000) Extensive homologous recombination among widely divergent TT viruses. J Virol 74:7666–7670
28. Rodriguez-Inigo E, Casqueiro M, Bartolome J, Ortiz-Movilla N, Lopez-Alcorocho JM, Herrero M, Manzarbeitia F, Oliva H, Carreno V (2000) Detection of TT virus DNA in liver biopsies by in situ hybridization. Am J Pathol 156:1227–1234
29. Ukita M, Okamoto H, Kato N, Miyakawa Y, Mayumi M (1999) Excretion into bile of a novel unenveloped DNA virus (TT virus) associated with acute and chronic non-A-G hepatitis. J Infect Dis 179:1245–1248
30. Ross RS, Viazov S, Runde V, Schaefer UW, Roggendorf M (1999) Detection of TT virus DNA in specimens other than blood. J Clin Virol 13:181–184
31. Itoh K, Takahashi M, Ukita M, Nishizawa T, Okamoto H (1999) Influence of primers on the detection of TT virus DNA by polymerase chain reaction. J Infect Dis 180:1750–1751
32. Nishizawa T, Okamoto H, Tsuda F, Aikawa T, Sugai Y, Konishi K, Akahane Y, Ukita M, Tanaka T, Miyakawa Y, Mayumi M (1999) Quasispecies of TT virus (TTV) with sequence divergence in hypervariable regions of the capsid protein in chronic TTV infection. J Virol 73:9604–9608