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Abstract: The non-coding control region (NCCR) of polyomaviruses includes the promoters for early and late genes, a transcription enhancer and the origin of DNA replication. Particularly virulent variants of the human pathogens BKPyV and JCPyV, as well as of simian virus 40 (SV40), occur in vitro and in vivo. These strains often harbour rearrangements in their NCCR, typically deletions of some DNA segment(s) and/or duplications of others. Using an SV40-based model system we provide evidence that duplications of enhancer elements, whether from SV40 itself or from the related BKPyV and JCPyV, increase early gene transcription and replicative capacity. SV40 harbouring subsegments of the strong cytomegalovirus (HCMV) enhancer replicated better than the common ‘wild-type’ SV40 in the human cell lines HEK293 and U2OS. In conclusion, replacing the SV40 enhancer with heterologous enhancers can profoundly influence SV40’s infective capacity, underscoring the potential of small DNA viruses to overcome cell type and species barriers.

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Transcription enhancers as major determinants of SV40 polyomavirus growth efficiency and host cell tropism

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The non-coding control region (NCCR) of polyomaviruses includes the promoters for early and late genes, a transcription enhancer and the origin of DNA replication. Particularly virulent variants of the human pathogens BKPyV and JCPyV, as well as of simian virus 40 (SV40), occur in vitro and in vivo. These strains often harbour rearrangements in their NCCR, typically deletions of some DNA segment(s) and/or duplications of others. Using an SV40-based model system we provide evidence that duplications of enhancer elements, whether from SV40 itself or from the related BKPyV and JCPyV, increase early gene transcription and replicative capacity. SV40 harbouring subsegments of the strong cytomegalovirus (HCMV) enhancer replicated better than the common ‘wild-type’ SV40 in the human cell lines HEK293 and U2OS. In conclusion, replacing the SV40 enhancer with heterologous enhancers can profoundly influence SV40’s infective capacity, underscoring the potential of small DNA viruses to overcome cell type and species barriers.
activating sequences and remove inhibitory ones. Interestingly, the genuine SV40 enhancer can be substituted with enhancers from unrelated viruses or from cellular genes, as was shown by the so-called ‘enhancer trap’, a selection system that utilizes an SV40 genome lacking the 72 bp repeats and adjacent enhancer sequences to regain infectivity by incorporating heterologous enhancer-active DNA segments (Günther et al., 2012; Weber et al., 1984). In the current work we have focused on the enhancer segments of SV40, BKPyV, JCPyV and of human cytomegalovirus (HCMV) for their ability to alter the host range of SV40, thus expanding on preliminary findings that a synthetic enhancer assembled from transcription factor binding sites can facilitate SV40 early gene expression and DNA replication in human embryonic kidney-derived cells (Günther et al., 2012). These studies are important since dual infections involving BKPyV, HCMV and SV40 have been reported in immunosuppressed transplant patients (Li et al., 2002; Nada et al., 2005). Moreover, co-infections and subsequent rearrangements can contribute to a broader cell and host tropism, at least in vitro (Henriksen et al., 2014; Kristoffersen et al., 1997; Myhre et al., 2010), and possibly support interaction, adaptation and pathology of polyomaviruses in new species including humans (Rinaldo & Hirsch, 2013).

First we tested two major forms of SV40: the archetypal one with only one 72 bp enhancer segment and the laboratory 776 strain with two copies. Since the SV40 archetype grew almost as fast as the wild-type in monkey kidney CV-1 cells (not shown), both viral forms were tested by competition in a co-transfection experiment. Even if the archetype was initially present in fourfold excess, it was swiftly overtaken by the laboratory strain such that in a second round of mixed infection, seeded with an aliquot of culture supernatant from the first round, only the laboratory strain with two 72 bp repeats was detectable (Fig. 1a). This result indicates that duplication of an active subsegment can confer a robust competitive advantage over the shorter form, despite the fact that there is, in principle, redundancy in the information content of enhancers (Schaffner et al., 1988).

Besides the 72 bp segment, which occurs in one or two copies, the SV40 promoter contains three imperfect repeats of 21 bp, each harbouring two binding sites for the transcription factor Sp1. Viral growth efficiency in CV-1 cells was reduced when only one or two copies of this 21 bp segment were present [Fig. 1b; see also Barrera-Saldana et al. (1985)]. In the same setting we also tested NCCR enhancer segments that had become repeated in strains of BKPyV and JCPyV upon their adaptation to growth in cell culture. SV40 lacking its own 72 bp repeats was reconstituted with and the efficiency of virus propagation was monitored. As is evident in Fig. 1b, the time to lysis was less for viruses containing repeated enhancer segments, in support of the model that this usually results in faster virus growth. The relatively weak activity of archetypal, non-repeated enhancers in SV40, BKPyV and JCPyV must nevertheless be of biological relevance - it probably helps these viruses to remain under the radar in immunocompetent hosts.

We also tested the different repeat numbers for their transcriptional efficiency. For this we used the versatile globin gene-based reporter system (Westin et al., 1987). Enhancer segments were inserted upstream of the reporter’s TATA box and transcript levels determined by the S1 nuclease assay. Note that reporter and reference genes do not replicate in transfected cells, which precludes possible confounding effects due to template copy number variation. The assay also indicates the location of the transcription start from the genuine reporter cap site and thus would reveal an altered transcription initiation, for example from within the enhancer. Three cell lines were tested: monkey CV-1, human embryonic kidney-derived HEK293, and mouse fibroblast-type Dko7 cells (Fig. 1c–e). In CV-1 cells, the number of 21 bp promoter segments and 72 bp enhancer SV40 repeats correlated with transcriptional activity; the BKPyV and especially the JCPyV repeats were weakly active (Fig. 1c). Of note, while transcriptional activity is clearly correlated with viral growth, the relationship did not appear to be linear; SV40 with BKPyV repeats grew better in CV-1 cells than would be expected from the transcript quantification (Fig. 1b vs c). This might indicate a contribution of SV40 sequences in the viral growth assay (see also below). In HEK293 cells, the BKPyV enhancer repeats were highly active and repeat numbers correlated well with transcriptional activity; even a single copy outperformed the SV40 enhancer, which was only poorly active in these cells (Fig. 1d). Given the strong activity of BKPyV enhancer repeats in HEK293 cells, we wondered whether SV40 with two or three BKPyV repeats would be able to productively multiply in these cells. To this end, cloned viral genomes were liberated by restriction digestion and transfected. After two, four and six days, the supernatant medium was analysed for viral load. As shown in Fig. 1f, in human cells the SV40-BKPyV recombinants yielded almost two orders of magnitude more virus than the wild-type SV40 (2×72 bp), again indicating that the repeated enhancer segments of the (rearranged) BKPyV Dunlop strain work well in HEK293 cells. Our findings also suggest that the BKPyV enhancer cooperates well with the SV40 early promoter. In this context we note that archetypal BKPyV neither produces T antigen nor replicates in HEK293 cells unless large T antigen is provided in trans in modified, so-called HEK293TT cells (Broekema & Imperiale, 2012). Furthermore, the archetypal BKPyV early promoter is weak even in natural host cells like human kidney RPTECs - most likely because it contains only a single Sp1 site compared to six in the SV40 promoter (Bethge et al., 2015).

In the next set of experiments, we tested the ability of the enhancer of the immediate early-1 gene of HCMV for its ability to promote SV40 growth, relative to the performance of the genuine SV40 enhancer. The HCMV enhancer, unlike the one of SV40, is strongly active in a great variety of cells and thus widely used in biotechnology for protein production in mammalian cells. A genomic
Fig. 1. Effect of duplication of enhancer segments on viral growth. (a) Growth competition between SV40 harbouring one 72 bp enhancer segment (archetype) vs two 72 bp segments ('wild-type' lab strain). Top, schematic of the control region for early transcription in the two strains tested. The cloned SV40 genomes containing 1 \times 72 \text{ bp} (archetype; light orange bar) or 2 \times 72 \text{ bp}
HCMV segment harbouring the enhancer was fragmented by sonication, mixed with an enhancerless linear SV40 genome and transfected into CV-1 cells. Using the ‘enhancer trap’ selection system (Fig. 2b) (Boshart et al., 1985; Günther et al., 2012; Weber et al., 1984), we obtained ten chimeric SV40-HCMV viruses, containing independent, but overlapping, enhancer inserts of various lengths and orientations relative to early transcription (Fig. 2c). In separate infections of CV-1 cells, most of the clones grew well but the one with the shortest HCMV insert (no. 10) performed poorly in this and other experiments. In the monkey cells, however, SV40 multiplied faster than the most efficient SV40-HCMV recombinant clone 7 (Fig. 2d). To determine whether any of the SV40-HCMV clones could propagate in human cells, a serial competition experiment (similar to the one in Fig. 1a) was done with the following four human cell lines: HEK293 (embryonic kidney-derived), U2OS (osteosarcoma), HepG2 (hepatoma) and Hela (cervix carcinoma); monkey CV-1 cells were used as a control. We used HEK293, rather than the derived, widely used HEK293 T cells because the latter constitutively express SV40 T antigen, which would have confounded the results. An equimolar mix of recombinants was ensured by quantifying viral genomes from lysate supernatants. From each cell type, two dishes were infected: one received only the ten SV40-HCMV recombinants; in the other one SV40 was also included. After seven days, cells and supernatants were harvested and processed as indicated in Fig. 2e. DNA bands from the fourth round of selection were cloned and individual colonies were sequenced. Selection in HepG2 and HeLa cells was discontinued after the first round because no viral DNA was detectable. Interestingly, even though all of the recombinants harboured overlapping segments of the HCMV enhancer, the competition indicated some cell type preferences (Fig. 2f): in HEK293 cells, clone 6 emerged as the predominant virus; clone 1 was strongly represented in U2OS cells but played at the most a minor role in HEK293 cells. Unlike the situation in monkey CV-1 cells, SV40 was not able to compete in these human cells with the more efficient chimeric SV40-HCMV clones. Also in another experiment with human embryonic retinoblast-derived 911 cells, clones 5, 6, 7 and 9 replicated faster than SV40 (not shown). Thus, with the heterologous HCMV enhancer, SV40 readily multiplied in three of the five tested human cells (HEK293, U2OS and 911), in line with the concept that the transcription enhancer is a major determinant of SV40’s cell type and species specificity.

Taken together, these results underscore the functional plasticity of the enhancer elements in polyomavirus NCCR's, which can subvert the replicative restriction occurring in host cell types not primarily infected (secondary host cell tropism). The in vitro experiments here suggest that in the absence of a functional immune control, re-arrangements including enhancer recombinations in the case of co-infections might not only contribute to organ pathology, but enhance cross-species transmission.
Fig. 2. Overview of the SV40 enhancer trap and SV40-HCMV recombinants. (a) The enhancer of human cytomegalovirus (HCMV) (21). A conspicuous feature of this enhancer are multiple copies of binding sites for each of the transcription factors CREB (pink), NF-κB (yellow), NF-1 (blue) and Sp-1 (green). The TATA box of the HCMV immediate early-1 promoter is shaded in grey. (b) The SV40 enhancer trap. A defective SV40 genome lacking the enhancer (deletion between positions 99 and 294) can incorporate enhancer-active DNA segments inside the transfected host cell, which restores virus growth (Weber 1601).
et al., 1984). (c) Schematic view of the ten different HCMV inserts in SV40-HCMV recombinant clones. Note that in clone 5, a large internal region is deleted; clones 1 and 8 are slightly shorter than the full-length enhancer segment and differ from each other at their junctions with SV40 DNA. For simplification, spacers between the binding sites for the above-mentioned transcription factors (in a) are omitted. Arrows indicate the orientation of the inserts within the SV40-HCMV recombinants. (d) Growth of SV40-HCMV recombinants in monkey cells. The ten recombinant viruses were grown individually on CV-1 cells, and the day when half of the cells were dead was taken as the endpoint. Dark grey: time to 50 % lysis of cells. Light grey up to 20 days: no clear cytopathic effect in at least one dish (clone 10 nevertheless produced enough virus for the competition experiment shown in e and f). The error bars, indicating SEM, are derived from infections done in duplicate dishes. (e) Growth competition of SV40-HCMV recombinant clones. An equimolar mix of SV40-HCMV recombinant viruses from infected cell supernatants was used as starting material to co-infect human HEK293 and U2OS cells, as well as monkey CV-1 cells. After four rounds of serial infection whereby 100 µl aliquots of supernatant were transferred to a new dish, viral DNAs were isolated by the alkaline precipitationneutralization method and analysed further. (f) Agarose gel electrophoresis of viral DNAs. After each round of selection procedure, viral DNAs were recovered for fractionation by agarose gel electrophoresis. After the fourth round, DNA from the gel bands was isolated, cloned and sequenced. In HEK293 cells clone 6 became dominant at the expense of the others, whereas in U2OS cells clones 1 and 6 were co-dominant. In CV-1 cells, where DNA was only analysed after the first and the fourth round, SV40 wild-type had outcompeted the SV40-HCMV recombinants by the end of the selection procedure.

Here, transplant patients might represent a critical, under-estimated mixing vessel.

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REFERENCES

Banerji, J., Rusconi, S. & Schaffner, W. (1981). Expression of a β-globin gene is enhanced by remote SV40 DNA sequences. Cell 27, 299–308.

Barrera-Saldana, H., Takahashi, K., Vigneron, M., Wildeman, A., Davidson, I. & Chambon, P. (1985). Efficient propagation of human polyomavirus BK virus genome. J Virol 51, 458–469.

Gardner, S. D., Field, A. M., Coleman, D. V. & Hulme, B. (1971). New human papovavirus (B.K.) isolated from urine after renal transplantation. Lancet 1, 1253–1257.

Gosert, R., Rinaldo, C. H., Funk, G. A., Egli, A., Ramos, E., Drachenberg, C. B. & Hirsch, H. H. (2008). Polyomavirus BK with rearranged noncoding control region emerge in vivo in renal transplant patients and increase viral replication and cytopathology. J Exp Med 205, 841–852.

Gosert, R., Kardas, P., Major, E. O. & Hirsch, H. H. (2010). Rearranged JC virus noncoding control regions found in progressive multifocal leukoencephalopathy patient samples increase virus early gene expression and replication rate. J Virol 84, 10448–10456.

Günther, V., Strassen, T., Lindert, U., Dagani, P., Waldvogel, D., Georgiev, O., Schaffner, W. & Bethge, T. (2012). Simian virus 40 strains with novel properties generated by replacing the viral enhancer with synthetic oligonucleotides. J Virol 86, 3135–3142.

Henriksen, S., Tylde, G. D., Dumoulin, A., Sharma, B. N., Hirsch, H. H. & Rinaldo, C. H. (2014). The human fetal glial cell line SVG p12 contains infectious BK polyomavirus. J Virol 88, 7556–7568.

Ilyinskii, P. O., Daniel, M. D., Horvath, C. G. & Desrosiers, R. C. (1992). Genetic analysis of simian virus 40 from brains and kidneys of macaque monkeys. J Virol 66, 6353–6360.

Katinka, M., Yaniv, M., Vasseur, M. & Blangy, D. (1980). Expression of simian polyoma early functions in mouse embryonal carcinoma cells depends on sequence rearrangements in the beginning of the late region. Cell 20, 393–399.

Kristoffersen, A. K., Johnsen, J. I., Seternes, O. M., Rollag, H., Degre, M. & Traavik, T. (1997). The human polyomavirus BK T antigen induces gene expression in human cytomegalovirus. Virus Res 52, 61–71.

Lednicky, J. A. & Butel, J. S. (1997). Tissue culture adaptation of natural isolates of simian virus 40: changes occur in viral regulatory region but not in carboxy-terminal domain of large T-antigen. J Gen Virol 78, 1697–1705.
Lednicky, J. A., Arrington, A. S., Stewart, A. R., Dai, X. M., Wong, C., Jafar, S., Murphey-Corb, M. & Butel, J. S. (1998). Natural isolates of simian virus 40 from immunocompromised monkeys display extensive genetic heterogeneity: new implications for polyomavirus disease. J Virol 72, 3980–3990.

Li, R. M., Mannon, R. B., Kleiner, D., Tsokos, M., Bynum, M., Kirk, A. D. & Kopp, J. B. (2002). BK virus and SV40 co-infection in polyomavirus nephropathy. Transplantation 74, 1497–1504.

Lim, E. S., Reyes, A., Antonio, M., Saha, D., Ikumapayi, U. N., Adeyemi, M., Stine, O. C., Skelton, R., Brennan, D. C. & other authors (2013). Discovery of STL polyomavirus, a polyomavirus of ancestral recombinant origin that encodes a unique T antigen by alternative splicing. Virology 436, 295–303.

McNees, A. L., White, Z. S., Zanwar, P., Vilchez, R. A. & Butel, J. S. (2005). Specific and quantitative detection of human polyomaviruses BKV, JCV, and SV40 by real time PCR. J Clin Virol 34, 52–62.

Moreau, P., Hen, R., Wasylyk, B., Everett, R., Gaub, M. P. & Chambon, P. (1981). The SV40 72 base pair repair repeat has a striking effect on gene expression both in SV40 and other chimeric recombinants. Nucleic Acids Res 9, 6047–6068.

Myhre, M. R., Olsen, G. H., Gosert, R., Hirsch, H. H. & Rinaldo, C. H. (2010). Clinical polyomavirus BK variants with agnogene deletion are non-functional but rescued by trans-complementation. Virology 398, 12–20.

Nada, R., Sachdeva, M. U., Sud, K., Jha, V. & Joshi, K. (2005). Co-infection by cytomegalovirus and BK polyoma virus in renal allograft, mimicking acute rejection. Nephrology Dialysis Transplantation 20, 994–996.

Newman, J. S., Baskin, G. B. & Frisque, R. J. (1998). Identification of SV40 in brain, kidney and urine of healthy and SIV-infected rhesus monkeys. J Neurovirol 4, 394–406.

Ondek, B., Shepard, A. & Herr, W. (1987). Discrete elements within the SV40 enhancer region display different cell-specific enhancer activities. EMBO J 6, 1017–1025.

Padgett, B. L., Walker, D. L., ZuRhein, G. M., Eckoade, R. J. & Dessel, B. H. (1971). Cultivation of papova-like virus from human brain with progressive multifocal leuencephalopathy. Lancet 1, 1257–1260.

Reddy, V. B., Thimmappaya, B., Dhar, R., Subramanian, K. N., Zain, B. S., Pan, J., Ghosh, P. K., Celma, M. L. & Weissman, S. M. (1978). The genome of simian virus 40. Science 200, 494–502.

Rinaldo, C. H. & Hirsch, H. H. (2013). The human polyomaviruses: from orphans and mutants to patchwork family. APMIS 121, 681–684.

Rochford, R., Campbell, B. A. & Villarreal, L. P. (1987). A pancreas specificity results from the combination of polyomavirus and Moloney murine leukemia virus enhancer. Proc Natl Acad Sci USA 84, 449–453.

Schaffner, G., Schirm, S., Müller-Baden, B., Weber, F. & Schaffner, W. (1988). Redundancy of information in enhancers as a principle of mammalian transcription control. J Mol Biol 201, 81–90.

Schaffner, W. (2015). Enhancers, enhancers – from their discovery to today’s universe of transcription enhancers. Biol Chem 396, 311–327.

Schirm, S., Jiricny, J. & Schaffner, W. (1987). The SV40 enhancer can be dissected into multiple segments, each with a different cell type specificity. Genes Dev 1, 65–74.

Seif, I., Khoury, G. & Dhar, R. (1979). The genome of human papovavirus BKV. Cell 18, 963–977.

Sundsfjord, A., Johansen, T., Flagstad, T., Moens, U., Villand, P., Subramani, S. & Traavik, T. (1990). At least two types of control regions can be found among naturally occurring BK virus strains. J Virol 64, 3864–3871.

Watanabe, S., Soeda, E., Uchida, S. & Yoshiike, K. (1984). DNA rearrangement affecting expression of the BK virus transforming gene. J Virol 51, 1–6.

Weber, F., de Villiers, J. & Schaffner, W. (1984). An SV40 "enhancer trap" incorporates exogenous enhancers or generates enhancers from its own sequences. Cell 36, 983–992.

Westin, G., Gerster, T., Müller, M. M., Schaffner, G. & Schaffner, W. (1987). OVEC, a versatile system to study transcription in mammalian cells and cell-free extracts. Nucleic Acids Res 15, 6787–6798.

White, M. K., Safak, M. & Khalili, K. (2009). Regulation of gene expression in primate polyomaviruses. J Virol 83, 10846–10856.

Yu, G., Greninger, A. L., Isa, P., Phan, T. G., Martinez, M. A., de la Luz Sanchez, M., Contreras, J. F., Santos-Preciado, J. I., Parsonnet, J. & other authors (2012). Discovery of a novel polyomavirus in acute diarrheal samples from children. PLoS One 7, e49449.