Supplementary data. Methodology of adenovirus and herpesvirus PCR assays.

Adenovirus PCR

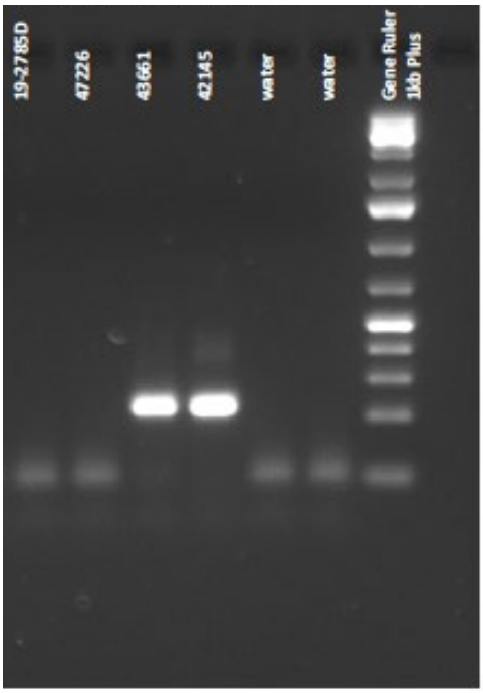
Extracted DNA from 25 mg of formalin-fixed, paraffin-embedded hedgehog lung: paraffin-embedded tissue (2 × 25 μm sections) were dewaxed with 1 mL of xylene 3 times and washed with 1 mL of 100% ethanol, 1 mL of 90% ethanol, and 1 mL of 70% ethanol. The pellet was resuspended in 95 μL of H_2O. DNA extraction and purification were performed (ZR genomic DNA tissue miniprep kit, Zymo Research), according to the manufacturer's instructions using 100 μL of DNA elution buffer and stored at −20°C until needed.

Degenerate PCR

Nested PCR amplification of a partial sequence of the adenoviral DNA polymerase gene was performed. The 25-μL first-round reactions contained HotStarTaq Plus master mix (Qiagen), 2.5 μL of extracted DNA, 0.2 μM concentrations for each primer (forward primer, polFouter [5′-TYMGVGGVGGBMGVTGYTAYCC-3′], reverse primer, polRouter [5′-GTRGCRAANSWSCCRTASAGGGCRTT-3′]). The mixtures were amplified with an initial denaturation at 95°C for 5 min followed by 40 cycles at 94°C for 30 s, 50°C for 30 s, and 72°C for 30 s. There was a final extension at 72°C for 10 min. For the second round, 1 μL of product from the first-round reaction was used for fully nested PCR with forward primer polFinner (5′-GTBTATGAYATHTGYGGSATGTATGC-3′) and reverse primer polRinner (5′-CCABYYKCKGTTRTGHARVGTRA-3′) or semi-nested using either primer pairs polFouter/polRinner or polFinner/polRouter. Second-round reactions were amplified under the same conditions as the first round. Primers are modified from those reported previously.[3]

Herpesvirus PCR

A pan-herpesvirus nested PCR assay was performed with degenerated consensus.[1,2] For the first PCR round, the reaction contained 12.5 µL of GoTaq Green master mix (Promega), 0.25 µL (of 20 µM) of each primer (DFA, ILK, and KG1), 6.75 µL of DEPC-treated water, and 5 µL of DNA sample. One µL of the amplified product was added to the second PCR mix with 0.25 µL (of 20 µM) of primer TGV and IYG. The cycling protocol for the first and second PCR corresponds to a published protocol.[1] An ovine herpesvirus 2–positive DNA extract from cattle brain tissue served as positive control. PCR products were analyzed by electrophoresis in 1% (w/v) agarose gels.



26324, 42145, and 43661: positive controls

47226: negative control

19-2785D: hedgehog lung tissue

References

1. Ehlers B, et al. Detection of new DNA polymerase genes of known and potentially novel herpesviruses by PCR with degenerate and deoxyinosine-substituted primers. Virus Genes 1999;18:211–220.

2. VanDevanter DR, et al. Detection and analysis of diverse herpesviral species by consensus primer PCR. J Clin Microbiol 1996;34:1666–1671.

3. Wellehan JF, et al. Detection and analysis of six lizard adenoviruses by consensus primer PCR provides further evidence of a reptilian origin for the atadenoviruses. J Virol 2004;78:13366–13369.