98

Selection of Woodchuck Hepatitis Virus Resistant to 3TC due to a Mutation in the Highly Conserved Polymerase FLLA Region in a Lifetime Woodchuck Antiviral Trial.

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We recently demonstrated that treatment with 3TC initiated early in the course of chronic WHV infection significantly inhibited hepatocarcinogenesis in a lifetime antiviral trial in chronically infected woodchucks. Since virus recrudescence in serum of some woodchucks treated with 3TC occurred, we examined twelve coded woodchuck plasma samples, six control samples and six treated with 3TC, obtained at 80 weeks. Of the six 3TC treated samples, three came from woodchucks with high levels of viral recrudescence in plasma as determined by Southern blotting. A DNA fragment containing domains A-E of the polymerase was isolated by PCR for each sample and analyzed by automated nucleotide sequencing. Surprisingly, all twelve samples were wild-type at the conserved YMDD motif in domain C associated with 3TC resistance in HBV. Four of the six 3TC treated woodchuck samples showed a mixture of the wild-type A (GCT) and the mutant T (ACT) at the conserved amino acid residue 566 (FLLA) in domain B. Interestingly, this change is associated with the amino acid 526 (FLLA) in HBV where 3TC and famciclovir selects for a change from L to M. In the woodchuck, the A to T change in the *pol* region results in a mutation of the surface protein from amino acid T (TGG) to an opal codon (TGA) which terminates the polypeptide. This transitional mutation creates a truncation in the surface protein forming a product reduced by 54 amino acids. The significance of these findings in relation to HBV pathogenesis and drug-resistance will be discussed.

99

Effects of HPMPC on Inhibition of HPV-16 Transformed Cell Proliferation

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(S)-1-(3-hydroxy-2-phosphonylmethoxy-propyl)cytosine (HPMPC) is a nucleoside phosphonate analog known to inhibit herpesvirus DNA polymerase. We have demonstrated that this compound can suppress proliferation of cells infected with human papillomavirus (HPV), which does not possess a viral DNA polymerase. We have shown that HPMPC induces an apoptotic response in HPV-16 transformed cells, indicating that this analog is cytotoxic rather than cytostatic. In order to further elucidate the mechanism of cell growth inhibition, we measured changes in cell cycle indicator/regulator expression and thymidine incorporation following treatment with HPMPC. HPMPC treatment reduced WAF1 (p21) levels, independently of p53, while augmenting expression of proliferating cell nuclear antigen (PCNA). However, in comparison to controls, HPMPC treated cells display a decrease in thymidine incorporation, indicating an inhibition of host DNA polymerase activity. Cell cycle analysis has revealed that HPMPC treated HPV-transformed cells are driven from G1 into the S-phase, but do not continue onto G2/M. The data suggest that an apoptotic response is initiated in HPV transformed cells trapped in S-phase.

100

Regulation by Human Papillomavirus E2 Proteins and Applications to Antiviral Drug Discovery

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Human papillomaviruses (HPVs) cause anogenital warts and other epithelial diseases and are linked to cervical cancer. The HPV E2 gene encodes a DNA-binding transcription factor. A systematic comparison of transcriptional activation by HPV E2 proteins revealed that the E2 proteins from HPVs linked to cervical cancer ("high-risk" HPV-16 and HPV-18) are much more active than the E2 proteins from low-risk HPVs (HPV-6 and HPV-11). *In vivo* experiments using a number of different cell types as well as *in vitro* assays demonstrated that this difference was intrinsic to the proteins and did not result from divergent DNA-binding properties or altered protein levels. In order to determine whether the lower observed transcriptional activity of the E2 proteins from low-risk HPVs was a consequence of the particular HPV-6 and HPV-11 clones being used, we also analyzed the sequence of E2 coding regions in HPV-6-containing clinical isolates and the activity of the encoded proteins. We found that these E2 proteins, the sequences of which fell into two categories, were also of low activity. Our studies thus demonstrate that there are at least two functional categories of HPV E2 protein, and this categorization needs to be taken into account when designing antiviral assays using the E2 protein as a target.

101

Effect of Acyclic Nucleoside Phosphonates (ANPs) on the Proliferation of Adenovirus-transformed Cell Lines as Compared to Human Papillomavirus (HPV)-harboring Cell Lines

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We have shown that treatment with ANPs of cell lines harboring HPV resulted in a time-dependent inhibition of cell proliferation. This inhibitory effect is particularly more striking for cidofovir (HPMPC). A 34- to 75-fold decrease in the 50% cytotoxic concentration (CC_{50}) at day 7 (as compared to day 3) was observed with HPMPC for several HPV-positive cell lines. A 5- to 15-fold decrease in CC_{50} was noted for HPMPC at day 7 in tumor cell lines non-containing HPV, but not in normal human cells. These results suggest that the anti-HPV activity of HPMPC may be explained, at least in part, by an anti-proliferative effect on rapidly proliferating cells and HPV might enhance the sensitivity of the cells to HPMPC due to an interaction of the viral transforming proteins with products of tumor suppressor genes. We have now evaluated the effects of the ANPs and other antiviral and antitumor drugs on SV_{40}-transformed African green monkey kidney (COS-1 and COS-7) cells and adenovirus-transformed human embryo kidney (293) cells, since viral transforming proteins of these cells are also known to interact with products of tumor suppressor genes. Treatment of COS-1, COS-7 and 293 cells with ANPs resulted in inhibition of cell proliferation in function of time similar to that observed for HPV-positive cells. The CC_{50} of HPMPC varied from 14-22 µg/ml at day 3 to 0.55-0.88 µg/ml at day 7, while the CC_{50} of HPMPC for the corresponding non-transformed cells varied from 92 µg/ml (Vero cells, African green monkey kidney) and 128 µg/ml (HEL cells, human embryonic lung fibroblasts) at day 3 to 12 µg/ml (Vero cells) and 84 µg/ml (HEL cells) at day 7. These resulted in a selectivity index (ratio CC_{50} for non-transformed cells to CC_{50} for transformed cells at day 7 of 16 (COS-1 cells), 18 (COS-7 cells) and 95 (293 cells). The effect of ANPs on the interaction of viral transforming proteins with products of tumor suppressor genes is currently under investigation.