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Investigation of molecular mechanisms underlying the oncosuppressive activity of Parvoviruses H-1 and MVM

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In order to better understand parvoviral oncolytic effects, the molecular mechanisms underline virus-cells interactions have been investigated through three different approaches both in vivo and in vitro.

1. To account for the oncolytic activity of parvoviruses, a possible link was sought between, on the one hand, expression of the tumor-suppressing protein p53 and, on the other hand, cytotoxicity associated with H-1 virus infection. To this end, a wild-type (wt) p53 inducible system derived from an endogenous $p53^-$ hepatoma cell line was chosen for the present study. Wt p53 activity can be induced in these cells (designated 4P) upon addition the inducer, 4-OH tamoxifen. At the optimal concentration of 4-OH tamoxifen (750 nM), the induction of p53 expression was found to have no detectable effects on the growth properties of uninfected cells. This allowed the inducible system to be used to analyze the effects of p53 on cell killing by parvovirus H-1. Our data indicate that in this system, p53 expression was not associated with a significant change in the sensitivity of cells to the cytotoxic action of parvovirus H-1. Therefore, no definite conclusion can be drown concerning a possible influence of p53 on cell susceptibility to parvovirus infection. Indeed the cellular system tested can be distinguished by its unaffected growth in the presence of p53 and by its peculiar resistance to some DNA-damaging agents, and may not be representative of the normal functioning of p53.

2. The question as to the tissue-specificity and developmental regulation of the MVM P4 promoter was addressed by means of a transgenic approach. This is an important issue for the understanding of oncolysis, because promoter P4 directs the transcription unit encoding the parvoviral cytotoxic proteins (designated NS). Three transgenic mouse lines were studied, two in which the LacZ reporter gene was placed under the direct control of P4 (P4-LacZ construct), and one harbouring the (P4-NS-P38-LacZ) construct in which LacZ expression is driven by the NS-inducible parvoviral promoter P38. The P4-LacZ transgenic mouse lines were tested for P4 activity in vivo. In both cases, the P4 promoter was found to remain silent during embryogenesis. In order to confirm and further investigate the possible reasons for such a lack of transgene expression, 27 primary cell cultures were prepared from embryos derived from all three transgenic mouse lines. Normal 3T3 cell lines and their SV40 transformed derivatives were further established from these primary cell cultures. The various cell lines allowed P4 promoter activity to be measured in vitro both qualitatively (Bluo-gal staining) and quantitatively (chemiluminescent reporter assays). Again, the P4 promoter was found to be silent in both normal and SV40-transformed 3T3 cells. Two lines of evidence

suggested that the transgenes were present. Firstly, a positive signal was obtained by DNA hybridization; secondly, NS1 proteins produced after infection of P4-NS-p38-LacZ transgenic cell cultures with MVM led to activation of the P38 promoter and expression of the reporter gene. Other observations suggest that our failture to detect P4 activation during embryogenesis is likely to be due to the strain of mice studied and/or the pattern of transgene integration in the animals tested. Our work shows that the proposed approach is feasible and should be extended to other mice.

3. New insights into the mechanisms by which parvovirus H-1 causes cell death was obtained in Hela cells and in SV40-transformed rat fibroblasts (P1) that were made inducible for the survival factors bcl-2 or hsp27. Through morphological analysis (Hoechst / PI dual staining), necrotic cell death was observed in P1 cells after H-1 virus infection. H-1 virus-induced cell killing occured via a bcl-2 and hsp27 insensitive pathway in the P1 cell system. Biochemical (PARP cleavage) and morphological (Hoechst / PI dual staining) analyses indicated that necrotic death also occured in Hela cells after H-1 virus infection, although some changes characteristic of apoptosis may be triggered at early times. H-1 virus infection of Hela cells was accompanied by rapid depletion of intracellular NAD stores. Stimulation of NAD production had no detectable effect on cell death. In contract, inhibition of NAD-consuming enzymes were found to shift the mode of death from necrosis to apoptosis in a fraction of Hela cells. These data show that parvoviruses can kill cells by both necrotic and apoptotic processes, and point to ADP ribosylation as a new parameter for the decision on whether cells will get involved in one or the other death pathway.

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