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The parvoviral capsid controls an intracellular phase of infection essential for efficient killing of stepwise-transformed human fibroblasts

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ABSTRACT

Members of the rodent subgroup of the genus *Parvovirus* exhibit lytic replication and spread in many human tumor cells and are therefore attractive candidates for oncolytic virotherapy. However, the significant variation in tumor tropism observed for these viruses remains largely unexplained. We report here that LuIII kills BJ-ELR 'stepwise-transformed' human fibroblasts efficiently, while MVM does not. Using viral chimeras, we mapped this property to the LuIII capsid gene, VP2, which is necessary and sufficient to confer the killer phenotype on MVM. LuIII VP2 facilitates a post-entry, pre-DNA-amplification step early in the life cycle, suggesting the existence of an intracellular moiety whose efficient interaction with the incoming capsid shell is critical to infection. Thus targeting of human cancers of different tissue-type origins will require use of parvoviruses with capsids that effectively make this critical interaction.

Introduction

Members of the rodent subgroup of genus *Parvovirus*, including H-1, LuIII, and Minute Virus of Mice (MVM), are potential candidates for oncolytic virotherapy of human cancer for several reasons. Firstly, these viruses are naturally S-phase dependent, so that they preferentially target host cell populations that are proliferating, while sparing those that are not. The rodent parvoviruses are also markedly oncoselective, displaying enhanced fitness and toxicity in many human cancer cell lines compared to their untransformed counterparts, although they show variable tropism between tumor types (Chen et al., 1986; Chen et al., 1989; Cornelis et al., 2004; Dupont et al., 2000; Guetta et al., 1990; Legrand et al., 1992; Mousset et al., 1994; Rommelaere and Cornelis, 1991; Van Hille et al., 1989). They show potent oncosuppressive properties in many *in vivo* models, including syngeneic mouse tumor models and human xenografts transplanted into immunocompromised animals (Dupressoir et al., 1989; Faisst et al., 1998; Raykov et al., 2007; Rommelaere and Cornelis, 1991; Shi et al., 1997; Toolan, 1967; Toolan et al., 1982), and can also induce both curative and protective immune responses in some immunocompetent rodent tumor models (Guetta et al., 1986; Geletneky et al., 2010). Importantly for the prospective use of these agents in virotherapy of human cancer, there is no human disease associated with any known member of this genus (Dupont, 2003; Rommelaere and Cornelis, 1991; Siegl, 1984). Finally, the small diameter of these viruses, approximately 20–25 nm, may facilitate intra-tumoral virion spread (Everts and van der Poel, 2005; Wu et al., 2001), while their rugged virion structure would allow them to survive protracted storage with minimal loss of potency.

In the present study we first illustrate that different parvovirus species vary in their ability to grow in and kill particular cells, and that different transformed human cells are variably susceptibility to virus-mediated killing, thus presenting hurdles for the rational targeting of human cancers. In order to further study this aspect of parvoviral target cell specificity, we have examined parvoviral oncoselectivity in stepwise-transformed human cells. In this approach, normal primary human cells are rendered tumorigenic in nude mice by the sequential retroviral transduction of transforming genes (Hahn and Weinberg, 2002), potentially allowing dissection of the contributions of individual oncogenes to specific steps in the parvoviral life cycle. Here we use one of the best characterized of these model systems (Hahn et al., 1999) to show that untransformed human fibroblasts are resistant to killing by a panel of rodent sub-group parvoviruses, but that the fully-transformed derivative, while still resistant to most of these viruses, is efficiently killed by the orphan parvovirus LuIII. This property maps to the LuIII coat protein gene, the product of which plays a critical role early in the establishment of infection of these transformed cells.

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Results

Rodent parvoviruses exhibit different host ranges in transformed human cells

In order to screen for the most effective virus:host cell combinations, we used a multi-well plate assay to monitor infection by a panel of five representative virus species that cluster phylogenetically with the rodent viruses within the genus *Parvovirus*. These include the prototype strain of the murine virus, MVMp, the two rat viruses H1 and H3 (the latter also known as Kilham Rat Virus), and two viruses of unknown animal origin, LuIII and TVX, originally isolated as contaminants of human cell lines (Hallauer et al., 1972). The viruses were grown in cell culture, purified and their genome concentrations determined by Southern blotting of alkaline gels probed with a conserved sequence oligonucleotide. Rapidly proliferating, sub-confluent monolayer cultures of cells in 24 well plates were infected at different input levels and stained for surviving cells 6 days post infection. This assay monitors several facets of viral infection, including acute killing at high multiplicity of infection (moi), as well as the virus' ability to proliferate and spread through the culture when infection is initiated at lower virus inputs. As shown in Fig. 1A, four of the viruses, MVMp, LuIII, H1 and H3 efficiently killed NB324K cells, while TVX had no detectable effect, even at high moi. In contrast, MVMp had no effect in HeLa cells, even at a high input level, while TVX completely destroyed the monolayer, even when infection was initiated at the lowest input multiplicity tested (32 genomes per cell, Fig. 1B). Since NB324K are papovavirus SV40-transformed newborn kidney cell line, whereas HeLa cells are derived from a papillomavirus-initiated cervical carcinoma, these two transformed human cell lines differ in many ways.

Complete stepwise oncogenic transformation of human fibroblasts dramatically enhances killing by parvovirus LuIII

Since it was unclear whether the differences in response to the parvovirus panel were due to the genetics of the host or tissue type, the nature of the transforming virus, or to stochastic differences that have arisen during transformation, we decided to examine the requirements for rodent parvovirus infection of transformed human cells in a more controlled fashion. To do this we tested the same panel of viruses for their ability to kill BJ primary human fibroblasts and their tumorigenic derivative, BJ-ELR, which were transformed by stepwise-addition of hTERT, the SV40 Early Region (SVER, which expresses both Large T Antigen (LT) and Small T Antigen (ST), and the activated proto-oncogene Ras^{V12} (Hahn et al., 1999). As shown in Fig. 1C, none of the five viruses had a detectable effect on the growth of the parental untransformed BJ cells at any of the input mois tested (up to 1000 genomes per cell). In contrast, two of the five viruses had pronounced effects on the survival of BJ-ELR monolayers (Fig. 1D). One of these, H1, only had observable effects at the highest input mois, but the other, LuIII, efficiently killed the majority of transformed cells down to an input of 64 genomes per cell, which represents a 16-fold lower initial moi than the maximum tested in this assay. Thus LuIII, and to a lesser extent by H1, are oncoselective for growth and killing in human fibroblasts since their infectivities are specifically enhanced by cellular alterations that drive oncogenesis. We also tested the lymphotropic strain of MVM, MVMi, for its ability to kill BJ-ELR, as well as three other MVM strains isolated as contaminants of commercial product streams (Garnick, 1996), but these all proved ineffective, indicating that BJ fibroblasts express a block to growth of MVM that is circumvented by LuIII.

Growth of MVM and LuIII are both enhanced by transformation, but only LuIII can expand effectively in transformed BJ cells

To better understand the phenotypes of MVMp and LuIII observed in the killing assay, we used an expansion assay, which measures the

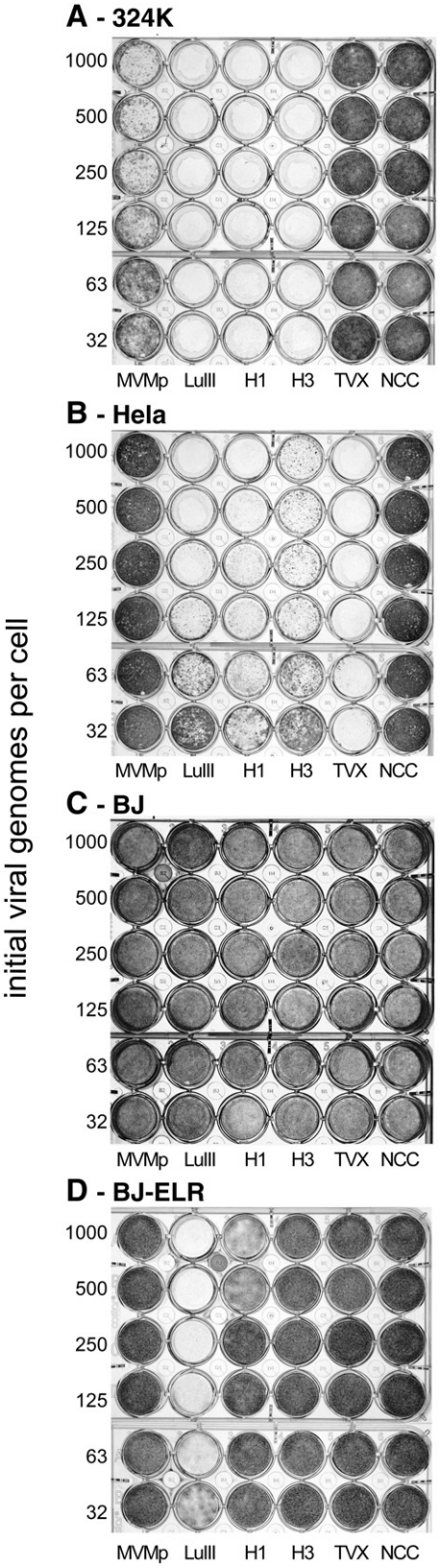

Fig. 1. Killing of transformed and normal human cells by a panel of five parvoviruses. Panel A: SV40-transformed new-born human kidney cells, NB324K, were seeded in 24-well plates at low density and infected at different multiplicities with genome-titered purified viral stocks of representative isolates of different species of *Parvovirus*, as indicated. Monolayers were incubated, fixed and stained as described in Materials and methods. The right-hand column represents the normal cell control (NCC). Panel B: HeLa cervical carcinoma cells; Panel C: BJ human fibroblasts; and Panel D: stepwise-transformed BJ-ELR (BJ + hTERT + SVER (LT + ST) + Ras) cells, respectively, were analyzed for viral killing as described for panel A.