Molecular Pathways: Rodent Parvoviruses—Mechanisms of Oncolyis and Prospects for Clinical Cancer Treatment

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Abstract

Rodent parvoviruses (PV) are recognized for their intrinsic oncotropism and oncolytic activity, which contribute to their natural oncosuppressive effects. Although PV uptake occurs in most host cells, some of the subsequent steps leading to expression and amplification of the viral genome and production of progeny particles are upregulated in malignantly transformed cells. By usurping cellular processes such as DNA replication, DNA damage response, and gene expression, and/or by interfering with cellular signaling cascades involved in cytoskeleton dynamics, vesicular integrity, cell survival, and death, PVs can induce cytostasis and cytotoxicity. Although productive PV infections normally culminate in cytolysis, virus spread to neighboring cells and secondary rounds of infection, even abortive infection or the sole expression of the PV nonstructural protein NS1, is sufficient to cause significant tumor cell death, either directly or indirectly (through activation of host immune responses). This review highlights the molecular pathways involved in tumor cell targeting by PVs and in PV-induced cell death. It concludes with a discussion of the relevance of these pathways to the application of PVs in cancer therapy, linking basic knowledge of PV–host cell interactions to preclinical assessment of PV oncosuppression.

Background

Over the past 3 decades, clinical research conducted with a view to optimizing multimodal therapeutic strategies has led to an ~20% increase in the 5-year survival rate of cancer patients [from 49% survival for the 1975–1977 diagnosis period to 68% survival for the 2001–2007 period (1)]. However, to further improve the outcome of cancer treatment, new therapeutic approaches are needed. In this regard, replication-competent oncolytic viruses (OV) appear to be promising. OVs are characterized by their ability to infect, propagate in, and kill tumor cells while sparing nontransformed cells, and thereby prime an anti-tumor immune response. Some of these agents [e.g., vesicular stomatitis virus, Newcastle disease virus, reovirus, and parvovirus (PV)] show an inherent preference for replicating in cancer cells due to their dependence on neoplasia-associated features, including deficiency of IFN response and aberrant activation of permissiveness factors. Investigators have engineered the oncotropism of other OVs (e.g., adenovirus, vaccinia virus, measles virus, and herpes simplex virus) by modifying or deleting viral genes or regulatory elements so as to make virus expression/replication entry conditional on the malignant phenotype. Furthermore, one can boost the oncosuppressive potential of OVs by arming them with transgenes/genetic motifs that confer an additional therapeutic effector function (e.g., immune-stimulation and prodruk activation) to recombinant viruses (2).

Oncolytic PVs

Rodent PVs are of particular interest in this regard because of their natural oncotropism, lack of preexisting antiviral immunity in most humans, and excellent safety profile (3). The suppressive activity of PVs against various rodent and human cancers has been documented in both in vitro systems and animal models (4, 5). PVs distinguish themselves by their dual oncolytic and immunostimulating activities, broad range of target tumors, ability to circumvent tumor cell resistance to conventional death inducers, and suitability for both local and systemic applications. Furthermore, their cytopathic effects can be recapitulated in vitro through the expression of a single parvoviral product, the nonstructural protein NS1, which functions as an onco-genic transformation-dependent toxin (3).

PVs belong to the genus Parvovirus of the family Parvoviridae. Among the PVs studied for their anticancer properties, 2 species—the minute virus of mice (MVM) and H-1PV, whose natural hosts are mice and rats, respectively—have been particularly characterized. PVs are nonenveloped icosahedral protein particles that are 24 nm in diameter and contain a linear single-stranded DNA (ssDNA) genome of ~5.1 kb. Their coding capacity is limited to the production of 2 capsid proteins (VP1 and VP2) and 5 nonstructural regulatory polypeptides (NS1, NS2, NS3, NS4A, and NS4B).

Expression of the NS1/NS2-coding...
genes is controlled by the early P4 promoter, and that of the VP1/VP2/SAT–coding genes is programmed by the late (NS1-inducible) P38 promoter (6).

To a certain extent, the restricted number (and size) of parvoviral proteins is compensated for by the multifunctionality of some of these products. This is exemplified by the NS1 protein, which exerts various functions ranging from viral DNA amplification and transcription regulation to cytotoxicity. These activities are timely regulated through NS1 posttranslational modification and/or subcellular distribution. In particular, the phosphorylation of distinct NS1 domains controls both enzymatic (ATPase and helicase) and nonenzymatic (DNA and protein binding) functions of the viral product, and varies during infection (7). In addition to being intrinsically multifunctional, NS1 interacts with a number of cellular partner proteins, further broadening the range of NS1-mediated activities (see below).

These features make PV strongly dependent on host cell factors. Of more importance, PVs require S-phase factors to start replication and viral protein production. Unlike tumor viruses, PVs are unable to promote the progression of quiescent cells into S-phase, despite their ability to enter quiescent cells (6). This dependency restricts PV infections to rapidly proliferating tissues and, together with other parameters (see Fig. 1), makes tumor cells preferential targets for PV replication and cytopathic effects.

Cell permissiveness and PV oncotropism

As depicted in Fig. 1, PV oncotropism is due in part to viral dependence on cellular replication and transcription factors whose synthesis and/or activity correlate with cell cycling and oncogenic transformation. Furthermore, the interplay between PVs and transformation-sensitive cell proteins raises the possibility that transformation might have an impact on late events of the PV life cycle, such as cytopathic effects and virus release. The presence of these factors is not sufficient, however, to ensure virus propagation. A restriction can occur, for instance, at the level of delivery of incoming viral genomes to the nucleus, a prerequisite to their conversion to double-stranded transcription templates (6). This may account for the heterogeneity of tumor cells with regard to permissiveness toward PV replication. It represents a target for optimization.

An additional barrier to the PV life cycle is created by the type I IFN–mediated antiviral response, of which PVs were recently shown to be both triggers and targets (8, 9). PV oncotropism may depend on whether many tumor cells become deficient in the IFN response, or on the capability of PVs to counteract this antiviral defense mechanism in transformed cells.

In addition to regulation of their synthesis, PV proteins are subject to functional modulations resulting from posttranslational modifications and alterations of subcellular distribution (7). These modulations may also contribute to oncotropism, because some of the cellular effectors proteins involved in this process [e.g., NS1-activating PRKCH/PKCα (10)] are overexpressed and/or altered in cancer cells (11). Cell-transformation–dependent modifications of the PV protein NS1 can thus be considered responsible for its specific toxicity in oncogene-transformed cells at concentrations that are innocuous to nontransformed cells (4).

An analysis of PV interactions with cell proteins (Fig. 2) reveals a striking interplay between PVs and cellular components of various metabolic pathways related to gene expression or DNA replication and repair. Some of these components are direct binding partners of PV proteins, as exemplified by the formation of complexes between NS1 and cellular transcription factors (TBP, TFIIA, and SP1/3) or replication factors [repli-actor protein A (RPA) (12–14)]. Others are known effectors and/or targets of the PV life cycle that are not found in complexes with PV proteins. Of interest, a number of these physical and/or functional partners of PVs get together in specialized areas of infected cells. In particular, many constituents of the cell DNA replication machinery (e.g., RPA, POLA1/POLα, PCNA, and RF-C) and DNA damage response (DDR, e.g., RPA-P32, γH2AX, NBS1-P, and ATM) are recruited to subnuclear PV replication centers called APAR bodies (15–17). This redistribution is expected to promote PV multiplication and contribute to host DNA synthesis shutoff. These various cell factors are established or potential actors in the PV life cycle, and may contribute to reshaping the intracellular environment to make it adequate for virus replication and spread. This cell-conditioning effect is illustrated by the phosphorylation chain of cellular protein kinases that is triggered by PV infection and leads to PDK-1–driven activation of PKCα and PKCη (18). As stated above, both members of the PKC family are essential for modifying the viral NS1 protein and enabling it to exert its replicative functions. This phosphorylation cascade forms a positive feedback loop that is thought to be initiated by the PV-induced nuclear export of PKCα, as a possible result of the interaction of the viral product NS2 with the cellular transporter XPO1/CRM1 (Fig. 1; ref. 7).

It is noteworthy that the major metabolic pathways that interconnect with PVs are subject to modulation through malignant transformation. In particular, overexpression of c-MYC and/or activated Ha-RAS renders normally resistant cells permissive toward PV propagation and NS1-induced killing (19). Both oncoproteins are known to have profound effects on the intracellular environment by dysregulating molecular pathways that are also involved in cell permissiveness toward PV infection. Factors that control transcription [E2F, ETS, ATF/CREB, and NF-YA (20–22)] or replication [CCNA1/cyclinA and DDR effectors (15–17, 23)] and protein kinases [PRKCH/ PKCη and PKB/AKT1 (ref. 10; Nüesch, unpublished data)] are among the cell proteins that act as modulators of the PV life cycle and belong to this signaling network, and whose expression or activation is affected by malignant transformation.

Cell disturbances and PV oncolysis

PV infection of permissive cells leads to shutoff of host macromolecule syntheses, notably causing early inhibition of cell DNA replication (6, 24), due to the sequestration of
components of the cell DNA replication machinery in viral replication centers (see above). Stalled replication forks (25) and ssDNA genomes of PV-related adeno-associated viruses (26) are known to activate the DDR. Another DDR-activating mechanism triggered by PVs is NS1-induced production of reactive oxygen species (ROS) causing DNA damage (27). PV-induced DDR signaling is mediated by activation of ATM but not ATR; it is characterized by phosphorylation of H2AX, NBS1, RPA32, CHK2, and P53; and results in accumulation of DNA repair proteins in viral replication centers (16, 17). Consequently, cell-cycle arrest after PV infection is observed at the S–G2 transition or G2 replication centers (16, 17). NS1 interacts with key cell cycle-regulatory proteins, such as CDK1, CCNB1/cyclinB1, CDKN1A/p21/Cip1, CDKN1B/p27/Kip1, and RLBP2/p130 (27, 29, 30). Arrested cells are proposed to offer a more favorable terrain for PV replication. Of interest, expression of the NS1 protein alone is sufficient to induce cell-cycle arrest (27, 29, 30), in keeping with the fact that NS1 is the major effector of virus cytotoxicity.

Parvovirus–Tumor Cell Interplay

Figure 1. PV-induced cell disturbances. Viral genome replication and gene expression take place in the nucleus and depend strictly on S-phase-associated cellular factors. Conversion of the viral ssDNA to a double-stranded transcription template is achieved by RFs under the control of cyclin A [1]. After conversion, S-phase (E2F) and transformation-sensitive (ATF/CREB, ETS, and NF-Y) TFs activate the PV early promoter P4, which controls expression of the nonstructural proteins NS1 and NS2 [2]. NS1 activates the late PV promoter P38, which programs capsid gene expression, and drives viral DNA amplification as an initiator protein and helicase (not illustrated in the figure). NS1 also interferes with cell survival at many levels. Direct interactions of NS1 with components of the DNA replication (RPA-1-3) and transcription (TFIIB, TFIIA, and SP5) machineries play a role both in viral DNA replication and transcription and in deregulating DNA/RNA metabolic processes in infected cells [3, 4]. Other virus protein–cell protein interactions, such as NS1-CKII-[5] and NS2-XPO1 [6], interfere with host cell signaling and nuclear export, respectively. These events affect the host cell dramatically by causing oxidative stress, DNA damage, cell-cycle arrest, cytoskeleton structure rearrangements, mitochondrial membrane depolarization, and/or lysosome permeabilization. Cell death ensues, and ectopic NS1 expression alone is sufficient to cause it. Productive PV infections are characterized by virus-induced cytosis followed by virus dissemination and subsequent rounds of infection. Besides providing permissiveness factors, transformed cells fail to mount an efficient anti-PV type I IFN response, which also contributes to the oncotypic properties of these agents. The oncoselectivity of PVs is thus attributed to 2 features of cancer cells: the presence of positive permissiveness factors and the inability to antagonize viral infection, as indicated with asterisks in the figure. ds, double-stranded; GS1, gelsolin; GTF2A/TFIIA, general transcription factor 2A; RF, replication factor; SP1/3, Sp1/3 transcription factor; TBP, TATA binding protein; TF, transcription factor; TPM2/5: tropomyosin 2/5; VP, PV capsid proteins; XPO1, exportin 1 (CRM1 yeast homolog).
surface. This transport seems to be important for the maturation of progeny particles and the eventual lysis of infected tumor cells (Bär et al., unpublished data).

Direct killing of tumor cells is not the only way oncolytic viruses can contribute to cancer suppression. That they might pass the job on to the immune system is actually expected, because a productive lytic viral infection of cancer cells should promote the display or release of both tumor-associated antigens and immunostimulating viral or cellular molecular patterns. Adoptive transfer, rechallenge, and immunodepletion experiments indicate that the host immune system takes part in PV-mediated oncosuppression (41, 42). The active role of PVs in inducing this anticancer immune response is further supported by the observation that PV infection of tumor cells enhances their ability to serve as an autologous vaccine (42, 43) and to stimulate both natural killer (44) and dendritic (45) cells with which they come into contact. Of note, the vesicular egress of progeny PVs may contribute to exposing intracellular tumor-associated antigens (38). Altogether, these data point to synergies between virus- and immune-cell–mediated oncolysis in PV-initiated tumor suppression.

Clinical–Translational Advances

Implications for cancer parvirotherapy

As discussed above, both activation of cellular helper functions and defects in cell defense mechanisms contribute to the permissiveness of cancer cells toward PV replication and toxicity. In contrast, the mildness of PV attack against normal tissues in adult animals results in clinically unapparent PV infections (46). This tolerance is also assumed to exist in humans and is the subject of a current clinical study. This trial involves patients with glioblastoma multiforme (GBM), the most malignant brain tumors against which standard treatments, including surgery and radiochemotherapy, are inefficient. Preclinical evidence of strong cytotoxic and oncosuppressive effects of H-1PV against glioma cells led to the launch in October 2011 of a first phase I/IIa study of H-1PV in patients with recurrent GBM [ParvOryx 01; ClinicalTrials.gov, identifier NCT01301430]. ParvOryx 01 is an open, noncontrolled, dose-escalation, single-center trial (47). H-1PV is administered before resection by either the intratumoral or the intravenous route, and again into the walls of the tumor cavity during tumor removal. The objectives of the trial are related primarily to local and systemic safety and tolerability, and secondarily to proof of concept.

Activation of (proto)oncogenes leads to changes in the intracellular environment that boost the parvoviral life cycle, as shown for c-myc (19). Overexpression of MYC drives malignant transformation in Burkitt lymphoma, medulloblastoma, and a large proportion of breast cancers with poor prognosis (48). In GBM stem cells, MYC is involved in regulating malignant progenitor cell differentiation, self-renewal, and tumorigenic potential (49). PV H-1PV efficiently suppresses these MYC-dependent tumors in preclinical models (refs. 50, 51; Lacroix, unpublished data). In response to PV infection, MYC expression is repressed in various cancer cell lines of different origins ( refs. 31, 52; Lacroix, unpublished data). These facts strongly suggest that cancers associated with MYC alterations are good targets for PV-induced oncolysis, and argue in favor of evaluating MYC as a predictive marker associated with tumor responsiveness to H-1PV treatment. In the absence of maternal immunity, PVs can infect rodent embryos, where they propagate in a variety of cell types while maternal tissues remain unaffected (46). This prompted us to hypothesize that malignant cells with embryonic features may show a similar permissiveness. Accordingly, neuroblastoma and pediatric medulloblastoma cell lines proved to be very susceptible to H-1PV killing in vitro, and we have observed suppression of xenotransplants in animal models (ref. 33; Lacroix, unpublished data). In addition to these hypothesis-based suggestions, needs-driven consideration of cancers with desolate prognosis should also influence the choice of tumor targets for subsequent PV clinical trials. In this regard, it is noteworthy that H-1PV synergizes with gemcitabine to kill pancreatic carcinoma cells and improves gemcitabine-based therapy of pancreatic tumors in animal models (53).

Within a given cancer entity, the heterogeneity of tumors calls for individualized treatments based on prior identification of patients most likely to respond. It is therefore important to discover biomarkers that can serve as predictors of individual tumor sensitivity to PV infection. For PDAC, a first candidate is the transcription factor SMAD4, which controls P4 promoter activity and hence nonstructural protein expression and toxicity (54). Other candidates should emerge from "omic" approaches that allow the correlative analysis of PV sensitivity and interactome status in tumors from large cohorts of patients.

Conclusions

Although PVs can cure cancers in animals, and raise new prospects for cancer therapy in humans, it is likely that their oncosuppressive capacity must be optimized before they can be used effectively in humans. The mere fact that some PVs have been isolated from human tumor xenotransplants indicates that their oncolytic activity is not always sufficient to arrest tumor growth and induce remission. This may be...
due to limitations on PV infectiousness, cytotoxicity, spread, and/or immunostimulation. A portfolio of second-generation therapeutics based on replication-competentPVs is under development to circumvent these limitations. Different complementary strategies might be used to achieve this goal. Virus variants with an enhanced ability to replicate lytically and spread in tumor cell populations are being isolated through natural selection or site-directed mutagenesis (55, 56). To minimize PV sequestration by normal tissues, PV capsids are first detoxified and then retargeted to cancer cells through the display of specific peptide ligands (57). The ability of PVs to act as adjuvants to boost the host antitumor response can also be increased by arming them with immunostimulatory molecular patterns (43). Furthermore, PVs can be combined with ionizing radiation (58), the cytostatic drug gemcitabine (53), the cytokine IFN-γ (41), or oncolytic reovirus (59) to achieve additive or even synergistic therapeutic effects. Indeed, the non-PV component of these combination treatments can be administered so as to minimize interference with PV replication and instead potentiate PV oncolytic or immunomodulating activities. These various improvements of replicative PV-based treatments should pave the way for future clinical testing of anticancer efficacy, after the hope for confirmation of H-1PV’s safety in the current clinical trial is achieved.

Disclosure of Potential Conflicts of Interest
J. Rommelaere and I. Lacrix received commercial research grants from Oryx GmbH & Co. KG. J. Rommelaere has an ownership interest (including patents) in the German Cancer Research Center. No other potential conflicts of interest were disclosed.

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Received February 28, 2012; revised March 29, 2012; accepted April 10, 2012; published OnlineFirst May 7, 2012.

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