(Re-)Engineering tumor cell-selective replicating adenoviruses: A step in the right direction toward systemic therapy for metastatic disease

An approach combining redundant controls to restrict the productive infection of adenoviruses to cells that are disrupted in the pRb pathway—a hallmark of human cancer—has resulted in a novel oncolytic virus that may be well suited for systemic administration to treat metastatic disease.

The use of viruses as oncolytic agents for tumor therapy was first described several decades ago, using a variety of viruses, including adenovirus, influenza virus, mumps virus, and Newcastle disease virus, among others, in a number of diverse cancer indications (reviewed by Mullen and Tanabe, 2002, and Kirn, 2000; Asada, 1974). These initial human trials met with limited durable responses, likely due to a number of reasons, including a poor understanding of molecular virology, inconsistent preparations of pure, well-characterized high-titer virus formulations, and inadequately controlled trials. Thus, this approach of "virotherapy" was largely abandoned and somewhat forgotten. However, the convergence in the ensuing years of the fields of molecular biology and virology provided a fertile ground for the understanding required to genetically engineer viruses and restrict their replication to cells with defects in cell cycle control pathways typical of cancer cells.

Frank McCormick and colleagues pioneered a novel paradigm for cancer therapy in 1996 (Bischoff et al., 1996) by describing an approach to restrict the productive lytic infection of adenoviruses to cells having inactivated p53, a defect that is ubiquitous among tumor cells. The "oncolytic" adenovirus ONYX-015—originally called *dl*1520 by its creators (Barker and Berk, 1987)—is deleted of the viral E1b 55K gene. In order to understand how ONYX-015 propagates selectively in tumor cells, a short primer in adenovirus biology is in order. The adenovirus early region one (E1) has 2 genes, E1a and E1b, which encode a number of proteins whose function it is to initiate the process of making the infected cell a good host, so that the virus can replicate its DNA, synthesize structural "coat" proteins, assemble progeny virus particles, lyse the cell, and start the same process all over again in neighboring cells. The adenovirus E1a proteins are the first viral gene expressed following infection, and have several functions, including activating the expression of all of the other viral early genes (i.e., viral proteins with enzymatic activity expressed prior to viral DNA replication), and mediating entry of the host cell into S phase through binding of pRb. In so doing, E1a liberates the E2F transcription factor from the pRb-E2F complex, and E2F subsequently activates the expression of multiple cellular genes required for progression into the cell cycle. As a consequence of E2F activation, apoptosis is induced; fortunately for adenoviruses, however, they evolved the E1b gene, which encodes proteins that suppress induction of both p53-dependent and independent apoptosis. In particular, the E1b 55K protein binds to p53 together with another viral protein expressed from the viral E4 gene, E4orf6, and exports p53 to the cytoplasm, where it is degraded. Yet another E4-encoded protein cooperates with E2F to activate the expression of Ad E2. Thus, the adenovirus E1 region proteins, together with a portion of the E4 proteins, inactivate the pRb and p53 tumor suppressor proteins in order to mediate entry of normal resting host cells into S phase and suppress induction of apoptosis, thereby promoting viral DNA replication and completion of the virus life cycle.

In formulating the oncolytic virus concept, McCormick reasoned that the pathways that are inactivated in normal cells infected by adenovirus (i.e., pRb and p53) are strikingly similar to those that are inactivated by tumor cells (McCormick 2000). Thus, adenoviruses deleted of the E1b 55K gene, like *dl*1520, should establish productive infection in cancer cells in which p53 was inactivated, but not in normal cells whose ability to regulate p53 was intact. Indeed, this was shown to be the case in comparing *dl*1520 productive infection between normal and several established tumor cell lines, and ONYX-015—as well as a new field of developing cancer cell-selective replicating viruses—was (re-)born.

In the development of ONYX-411 described in this issue of *Cancer Cell*, Leisa Johnson and colleagues (Johnson et al., 2002) have dramatically improved upon the concept of ONYX-015. In the process, they have created a highly sophisticated recombinant adenovirus that may make systemic administration of selectively replicating viruses for the treatment of metastatic malignancies a more realistic possibility. Productive infection of ONYX-411 was designed to be restricted to cells having defects in the pRb pathway, a phenotype common to nearly all human cancers (Hanahan and Weinberg, 2000). ONYX-411 replication was shown to occur at near wild-type Ad levels in multiple human cancer cell lines in vitro, but was essentially non-cytotoxic in several cultured primary normal human epithelial cells. The inability of ONYX-411 to establish productive infection in normal human cells was a result of an inability of the virus to mediate efficient entry of these cells into S phase. Notably, ONYX-015 was consistently observed to be attenuated in human tumor cells, as compared to ONYX-411 and wild-type Ad. This observation may be due to another function of the Ad E1B 55K protein, to facilitate selective nucleocytoplasmic transport of viral mRNA late in the infectious cycle.

How was the replication of ONYX-411 engineered to be so tightly controlled? By two means: first, by regulating the expression of both the viral E1a and E4 genes; and second, by constructing redundant controls into the expression and activities of these viral proteins. The expression of the Ad E1 and E4 genes in ONYX-411 is controlled by replacing the native viral promoters with the human E2F promoter. The E2F promoter has been shown by others to be selectively active in tumor cells in vivo, which typically contain "high free-E2F" levels, and its function is linked directly to the pRb status of the cell. Importantly, the conserved region (CR) 2 of E1a, which corresponds to its high affinity binding pRb binding portion that medi-

Table 1. A partial list of replication-selective viruses in development

Parent virus; name (if available); phase(s) of ongoing human clinical trials for replication-selective viruses	Disease indication	Virus replication-permissive tumor cell phenotype	Reference
Ad 2/5 (*dl* 309); ONYX-015; I–III	Head and neck cancer, colorectal liver metastases, advanced solid cancers	$p53^-$	1–3
Ad 2/5 (*dl* 309); ONYX-411	Cervical cancer xenografts (preclinical studies)	TSP (E2F), pRb^-	4
Ad5; 01/PEME; NA	Several cancer xenografts (preclinical studies)	$p53^-$	5
Ad 5; CG 7060; I–II	Early-stage prostate cancer	TSP (PSA)	6
Ad 5; CG 7870; I–II	Hormone-refractory prostate cancer	TSP (PSA, Probasin)	7
Ad5; CG 8900; NA	Hepatocellular carcinoma (preclinical studies)	TSP (AFP)	8
Ad5; Ad5-Δ24RGD; NA	Ovarian (preclinical studies)	pRb^-, $integrin^+$ cells	9
HSV-1; G207; I–II	Malignant glioma	Deficient PKR response, intracellular nucleotide pools	10
HSV-1/HSV-2; NV1020; I	Colorectal liver metastases	Not determined (safe in human vaccine trials)	11
NDV; PV701; I	Advanced solid cancers	Activated *ras*, deficient PKR response[a]	12
Reovirus; Reolysin; I–II	Malignant glioma, prostate, advanced solid cancers	Activated *ras*, deficient PKR response	13
VSV; NA; NA	Melanoma xenografts (preclinical studies)	Activated *ras*, deficient PKR response	14
Rhinovirus/poliovirus intergeneric recombinant; PV1(RIPO); NA	Malignant glioma (preclinical studies)	CD155 Poliovirus receptor; activated *ras*, deficient PKR response[a]	15
Measles (MV-Ed); I	Ovarian cancer	Activated *ras*, deficient PKR response[a]	16

NA: Not applicable. TSP: Tissue Specific Promoter, used in the construction of the replication-selective virus. Responsiveness of the TSP indicated.
[a]Possible mechanism for virus replication selectivity; actual mechanism not demonstrated
[1]Khuri et al. (2000). Nat. Med. *6*, 879–885; [2]Reid et al. (2001). Proc. Am. Soc. Clin. Oncol. *20*, 549a; [3]Nemunaitis et al. (2001). Gene Ther. *8*, 746–759; [4]Johnson et al. (2002) Cancer Cell *1*, this issue; [5]Ramachandra et al. (2001). Nat. Biotechnol. *19*, 1035–1041; [6]Rodriguez et al. (1997). Cancer Res. *57*, 2559–2563; [7]Yu et al. (1999). Cancer Res. *59*, 4200–4203; [8]Li et al. (2001). Cancer Res. *61*, 6428–6436; [9]Bauerschmitz et al. (2002). Cancer Res. *62*, 1266–1270; [10]Mineta et al. (1995). Nat. Med. *1*, 938–943; [11]Cozzi et al. (2001). FASEB *15*, 1306–1308; [12]Pecora et al. (2002). JCO *20*, 2251–2266; [13]Norman et al. (2000). J. Clin. Inves. *105*, 1035–1038; [14]Stojdl et al. (2000). Nat. Med. *6*, 821–825; [15]Gromeier et al. (2000). Proc. Natl. Acad. Sci. USA *97*, 6803–6808; [16]Grote et al. (2001). Blood *97*, 3746–3754.

ates release of E2F, is deleted in ONYX-411. Thus, the $CR2^-$ deleted E1A protein expressed by ONYX-411 in normal cells is largely unable to break up the pRB-E2F protein complex. The combination of a tumor-specific promoter at each end of the virus together with an E1a protein unable to mediate S phase entry was shown to be necessary to derive a virus with the degree of restriction to cancer cell lines that was observed with ONYX-411.

What clinical advantage could ONYX-411 confer as compared to the ONYX-015 prototype? Theoretically, ONYX-411 may be better suited for applications, like metastatic disease, requiring systemic virus administration. As the mechanism of selection for virus replication is operative post virus absorption, both normal and cancer cells are infected following intravenous virus (IV) administration. Thus, the therapeutic index of selectively replicating viruses can be defined by their ability relative to wild-type virus to establish productive infection in human cancer cells, as compared to normal primary cells. The therapeutic index for ONYX-411 was observed to be quite high, and in some cases it was 1000-fold less cytotoxic than wild-type Ad in normal cells. Yet, ONYX-411 consistently replicated to wild-type virus levels in multiple cancer cell lines. Based on observations in preclinical studies performed in mice receiving high IV doses of ONYX-411, including an absence of acute hepatotoxicity, and reduction of established subcutaneous tumors correlating with increased survival time, further testing, including possibly human clinical trials, is warranted.

As cancer is primarily a disseminated disease, new therapeutic agents must be able to provide durable systemic responses against metastatic disease originating from primary tumors of diverse origin. For a virus to have systemic activity, it must do three things effectively, including: (1) gain access to the tumors; (2) replicate and spread within the tumor; and (3) kill the tumor cells, either directly or indirectly. High doses (4×10^9 plaque forming units) of ONYX-411 given IV were shown to elicit no more acute toxicity in immunocompetent mice than PBS. Thus, since it is expected that comparatively higher doses would correlate with higher amounts of virus accessing tumor cells following IV administration, in terms of each of these three criteria, ONYX-411 would appear to be a significant improvement upon ONYX-015.

Are adenoviruses the optimal platform for tumor-selective replicating viruses? The encouraging successes of ONYX-015 in early- and mid-phase clinical trials, particularly when combined with conventional chemotherapy (Khuri et al., 2000; Reid et al., 2001)—as well

as the controversy over the requirement for a defective p53 pathway to support the replication selectivity of ONYX-015—stimulated the development of numerous additional oncolytic virus platforms. The controversy surrounding the inactivated-p53 requirement for ONYX-015 replication has been somewhat resolved through studies demonstrating that a subset of tumor cells contain a wild-type p53 gene, yet are functionally p53 inactive, due to inactivation of the "upstream" $p14^{arf}$, resulting in the accumulation of mdm2, which binds p53 and targets it for degradation, much in the same way as does the adenovirus 55K protein (McCormick, 2000; Ries et al., 2000). While improved conditionally replicative adenoviruses like ONYX-411 and others (Li et al., 2001; Ramachandra et al., 2001) have been developed, the question still remains whether oncolytic agents developed from other virus genera will be more efficacious for local-regional or systemic applications. Table 1 summarizes some other viruses in development. The properties of these viruses in terms of the expression of their cognate receptors on various tumor cell types, reproductive cycle time, yield, cytotoxicity, and selectivity cover a broad spectrum. Importantly, the issue of virus-specific immune responses will need to be addressed in order to repeatedly administer any of these viruses intravenously. Another important issue related to some of these viruses may be their possible safety risk; in several instances, viruses are being used without genetic modification—such as deleting critical virus regulatory genes—suggesting the possibility of in vivo selection of virulent phenotypes. For these or any oncolytic viruses to eventually gain FDA approval and become the standard of care will require continued refinement and testing, both in preclinical models and in safety and efficacy trials in humans. Nevertheless, the complete makeover that ONYX-015 has received in the design and development of ONYX-411 would appear to be a step in the right direction toward oncolytic viruses becoming useful therapies for the treatment of selected cancers.

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Senescence: a companion in chemotherapy?

Mouse lymphoma model points to an unsuspected role of drug-induced "senescence."

For decades we have been attempting to understand the basis of drug resistance manifested by so many cancers. Even tumors never previously exposed to anticancer agents often already show intrinsic drug resistance, suggesting that gene mutations driving tumor development do not automatically confer resistance to anticancer agents. In two recent papers, Lowe and collaborators (Schmitt et al., 2002a, 2002b) convincingly show that in a lymphoid mouse tumor model, drug resistance can be conferred not only by lesions in the apoptotic pathway but also, surprisingly, by mutations in the senescence pathway. The realization that an intact senescence pathway can contribute to the success of chemotherapy may have profound consequences for the treatment of cancer patients.

Several mechanisms can contribute to drug resistance. Cytotoxic drugs, at the moment still the backbone for treatment of disseminated cancer, almost invariably damage DNA or interfere with DNA replication or chromosomal segregation. One way cells can prevent killing by anticancer drugs is to expel the drugs from the cell by transporters. Even low amounts of these transporters substantially decrease the sensitivity of cells to cytotoxic drugs (Allen et al., 2000, and references therein). However, this does not explain why tumors often are inherently resistant to anticancer agents. One has to assume that this tolerance arises as a side effect of the genetic alterations that drive the tumorigenic process. Indeed, mutations interfering with apoptosis can contribute both to tumor growth and confer resistance to chemotherapy in a mouse lymphoma model (Schmitt et al., 1999). However, clinical practice teaches that tumors with clear defects in the apoptotic machinery are not neces-