

Targeting Cancer-initiating Cells With Oncolytic Viruses

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Recent studies in a variety of leukemias and solid tumors indicate that there is significant heterogeneity with respect to tumor-forming ability within a given population of tumor cells, suggesting that only a subpopulation of cells is responsible for tumorigenesis. These cells have been commonly referred to as cancer stem cells (CSCs) or cancer-initiating cells (CICs). CICs have been shown to be relatively resistant to conventional anticancer therapies and are thus thought to be responsible for disease relapse. As such, they represent a potentially critical therapeutic target. Oncolytic viruses are in clinical trials for cancer and kill cells through mechanisms different from conventional therapeutics. Because these viruses are not susceptible to the same pathways of drug or radiation resistance, it is important to learn whether CICs are susceptible to oncolytic virus infection. Here we review the available data regarding the ability of several different oncolytic virus types to target CICs for destruction.

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INTRODUCTION

Over the past 40 years, anticancer agents have entered clinical practice and improved survival for many patients with cancer. Unfortunately, even for patients who achieve remission following treatment with surgery, chemotherapy, radiation therapy, and some of the newer targeted inhibitors and antibodies, relapse often occurs months or years later. Hypotheses to explain this phenomenon include ideas such as tumor cell acquisition of resistance, suboptimal surgical debulking and inability of chemotherapy and radiotherapy to target all cancer cells within a given patient. The cancer stem cell (CSC) theory postulates that there are subpopulations of cells, also known as cancer-initiating cells (CICs), responsible for relapse.

The term "CSCs" was originally coined to describe features of these cells that are similar to bona fide normal stem cells.¹ Whether CSCs arise from normal stem cells is still under debate; their origin is likely to vary among different cancers. By definition, CSCs possess the shared properties with normal stem cells of self-renewal and pluripotency. Normal stem cells also express drug resistance genes, such as the ATP-binding cassette protein efflux pump ABCG2, which presumably provide these cells with a measure of protection from environmental toxins.² Similarly, many identified CSCs express such genes and are relatively resistant to chemotherapy and irradiation, making them prime candidates as the source of relapse.³ Because CSCs may not share other features with normal stem cells, and due to their uncertain origin, there is a growing preference to refer to them by the less controversial and more precise terms of CICs. Nevertheless, their role in producing daughter cells that constitute the bulk of a tumor is similar to

the role of normal stem cells in generating the bulk of an organ (or blood, in the case of bone marrow stem cells). Although considerably more study is required to faithfully classify, isolate, and characterize the biology and role of CICs, therapeutic targeting of these cells may reduce relapse rates and improve long-term outcome for patients with many types of cancers.⁴

The past decade has seen an explosion of research into the field of gene therapy and therapeutic, or so-called "oncolytic," viruses.⁵ Such viruses fall into broad categories of (i) wild-type animal viruses that do not typically infect human cells but are cytotoxic to human cancer cells, (ii) attenuated mutants of human viruses in which critical genes for virus replication that are dispensable in cancer cells have been deleted or mutated, and (iii) viruses that have been attenuated by serial passage in culture, such as most live virus vaccines. These agents hold much promise as they have been shown to be efficacious against malignant tissues, yet minimally toxic to their normal cell and tissue counterparts. Oncolytic viruses are effective against a wide variety of human cancers in preclinical models and encouraging results from clinical trials are beginning to accumulate. Novel methods of delivery, including cell-based schemes, appear to increase their ability to reach distant metastatic sites of disease, counteracting a major criticism that they will be useful only for localized disease.⁶ Oncolytic viruses may also be engineered to deliver therapeutic transgenes, thereby increasing their antitumor effects.

The question of whether oncolytic viruses are well suited to eliminate CICs has begun to be addressed. Oncolytic viruses seem like ideal candidates to target CICs because they are cytotoxic and are not subject to the typical mechanisms of drug resistance

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such as drug efflux pumps and defective apoptotic signaling.⁷ In addition, viruses may be engineered to express therapeutic transgenes that specifically target properties that CICs rely upon for self-renewal and cell division. Indeed, initial studies suggest that oncolytic viruses may be effective against and may be directed toward CICs.⁸ The focus of this review is to highlight recent studies using oncolytic viruses against CICs to determine whether oncolytic viruses are able to eradicate CICs and prevent tumor formation or relapse. We have separated our discussion of DNA viruses from RNA viruses because they exhibit fundamental differences in their life cycles that might impact their ability to kill CICs (DNA viruses replicate in the nucleus whereas RNA viruses replicate in the cytoplasm).

DNA VIRUSES

Herpes simplex virus

The majority of studies with herpes simplex virus (HSV)-derived mutants have been conducted in models of malignant brain tumors, partly because of HSV's neurotropism as well as the fact that these were among the first solid tumors in which CICs were identified. Initial studies of brain tumors mainly used serum-free basal medium supplemented with epidermal growth factor and fibroblast growth factor (EF medium) to culture surgical specimens.^{9,10} This medium, also referred to as neural stem cell medium, was originally designed for culture of neuronal stem cells to maintain their "stemness" properties *in vitro*.¹¹ When cultured in EF medium, primary brain tumor specimens form three-dimensional spheres and show many features resembling normal stem cells, including self-renewal, expression of stem cell markers such as CD133 and nestin, and multilineage differentiation. Most importantly, cells derived from these "tumorspheres" are able to form xenografts in mice that recapitulate their original morphological phenotype.^{10,12}

The HSV-1 *RL1* gene encodes ICP34.5, the "neurovirulence factor," which reverses the host cell's protein kinase R mediated shutoff of protein synthesis, thereby enabling efficient virus replication even in the presence of a robust interferon (IFN) response.^{13,14} Virus mutants deleted for *RL1* alone (e.g., HSV1716) or *RL1* combined with deletion of the *U_L31* gene that encodes the large subunit of ribonucleotide reductase, ICP6 (e.g., G207, OncoVex^{GM-CSF}), have proven safe in clinical trials by direct intratumoral injections in brain, melanoma and head/neck squamous cell carcinoma.¹⁵⁻²¹ While severely attenuating virus replication in normal postmitotic cells, lack of ICP34.5 expression also impairs their replication to some degree in cancer cells. Taking advantage of differential expression of nestin in glioma cells versus normal astrocytes, Kambara *et al.* restored ICP34.5 expression in an ICP34.5-deleted virus under the control of the nestin enhancer. This virus, rQnestin34.5, showed selective targeting to glioma cells.²² Nestin is also expressed in many brain tumor stem cells and its expression is decreased in differentiated cells. Otsuki *et al.* showed that primary glioma tumorspheres were effectively targeted by rQnestin34.5 compared to the ICP34.5-null control virus, rHSVQ1 (ref. 23). Moreover, pretreatment with the histone deacetylase inhibitor, valproic acid, augmented viral propagation and antitumor efficacy in glioma cells including primary sphere cells. The authors found that valproic acid inhibited the IFN response and significantly

downregulated IFN-responsive antiviral genes following HSV-1 infection. Valproic acid pretreatment also counteracted exogenously applied IFN β -mediated inhibition of viral propagation. Interestingly, IFN β treatment inhibited rQnestin34.5 replication by >99.99% in primary sphere cells while showing far less effects in other tested glioma cultured lines. Similarly, Zhang has found that breast CICs isolated from cell lines are somewhat resistant to infection with an oncolytic HSV-2 mutant, but could be made sensitive with addition of the histone deacetylase inhibitor, trichostatin A (X. Zhang, Baylor College of Medicine, personal communication, 6 Jun 2009). Taken together, these findings suggest that an intact IFN response may be a unique feature of CICs compared with bulk cancer cells.

Using the same rQnestin34.5 virus, Mahller *et al.* showed that CICs from a neuroblastoma cell line, LA-N-5, were susceptible to infection by oncolytic HSV-1 (ref. 8). Neuroblastoma is a childhood cancer thought to arise from the embryonic neural crest.²⁴ LA-N-5 cells cultured in EF medium as tumorspheres were enriched for expression of CD133 and ABCG2 and more resistant to doxorubicin compared to the serum-grown bulk cultures. Spheres derived from LA-N-5 and two other neuroblastoma cell lines, IMR-32 and CHP-134, showed multilineage differentiation indicating pluripotency of neuroblastoma tumorspheres. *In vitro* cytotoxicity and viral production assays confirmed that both LA-N-5 bulk cells and tumorspheres were sensitive to nestin-targeted HSV-1 infection. In addition, mice inoculated with LA-N-5 bulk cells preinfected with rQnestin34.5 showed no flank tumor formation for >2 months whereas mice injected with saline or control virus-treated cells showed rapid tumorigenesis.

Because these studies used established cell lines, it is important to determine whether similar results are found in primary human CICs. One source of such CICs is from the bone marrow of patients with neuroblastoma, as reported by Hansford *et al.*, obtained by culturing bone marrow aspirates in EF medium.²⁵ The resulting sphere-forming cells were highly tumorigenic, requiring as few as 10 cells to initiate tumor growth in immunocompromised mice. In collaboration with Hansford *et al.*, we have found two of three primary CICs to be as susceptible as LA-N-5 cells to oncolytic HSV infection (P.-Y. Wang and T.P. Cripe, unpublished results). Reasons for resistance of the third culture are under investigation.

In invasive glioma models, Wakimoto *et al.* showed that the HSV-1 mutant, G47 Δ (ICP6⁻, γ_2 34.5⁻, α 47⁻), effectively targeted glioblastoma (GBM) CICs.²⁶ G47 Δ is derived from G207 (ICP6⁻, ICP34.5⁻) with the additional deletion of the α 47 coding sequence and the *U_S11* promoter, which places expression of the *U_S11* gene under the control of the α 47 promoter. Expression of the normally late-expressed *U_S11* gene under the immediate-early α 47 promoter partially compensates for the lack of ICP34.5 and enhances viral growth in infected cells.²⁷ Four GBM surgical specimens were cultured *in vitro* as tumorspheres and confirmed to exhibit CIC characteristics (CD133 and nestin expression, differentiation potential, and tumorigenicity in an orthotopic xenograft model).²⁶ Tumorigenicity between the different GBM cells appeared to correlate with CD133 expression. Only 50 GBM8 sphere-derived cells (92.8% CD133⁺) were required to consistently form tumors in mice, whereas nearly 5×10^4 GBM8 cells cultured in FCS condition

(<20% CD133⁺) and 5×10^3 GBM4 sphere cells (38.1% CD133⁺) were required to create tumors *in vivo*. In comparison with wild-type and different HSV-1 F strain mutants, G47 Δ showed less anti-tumor efficacy in GBM spheres than wild-type and ICP6-deficient F Δ 6 but significant more than ICP34.5 mutants (G207 & R3616), which had a minimal effect on cell survival. G47 Δ targets both CD133⁺ and CD133⁻ GBM cells, and infected GBM cells were unable to form secondary spheres *in vitro*, suggesting GBM CICs are susceptible to virus infection. A single intratumoral injection of G47 Δ prolonged survival of mice bearing CIC-derived tumors. Similarly, recent studies by Friedman *et al.* suggest glioma progenitor cells are sensitive to killing by HSV ICP34.5-defective mutants, but only if the cells express sufficient amounts of the major HSV receptor, nectin-1 (ref. 28). It is of interest to note that some CICs have been shown to have relatively intact and even hyperactivated DNA repair mechanisms,²⁹ a property thought to be responsible for their relative resistance to chemotherapy and irradiation, and activated DNA repair pathways enhance replication of HSV mutants.³⁰

Adenovirus

In order to replicate, adenovirus (Ad) promotes entry of cells into the G₁ phase of the cell cycle by binding Rb via the immediate-early protein E1A and releasing the transcriptional factor E2F.³¹ Thus, Ad is capable of infecting both dividing and nondividing cells. Because many tumor cells harbor defects in the Rb/p16 pathway, mutant Ads with a 24 base pair deletion of the E1A Rb binding site (Δ 24) showed tumor selectivity as viral replication was abrogated in normal cells with intact Rb/p16 (refs. 32,33). Most Ad serotypes, including commonly used Ad5, enter into cells through their viral fiber knob binding to the host cell surface coxsackie-Ad receptor,³⁴ which is highly expressed on normal epithelial cells but lacking in many tumor cells.³⁵ Modification of the viral capsid to change the Ad tropism has been a common strategy to overcome the lack of coxsackie-Ad receptor in tumor cells.

Eriksson *et al.* showed capsid-modified E1A mutated Ads, Ad5/3- Δ 24 (ref. 36), which uses the Ad serotype 3 receptor that is highly expressed in tumor cells, and Ad5.pk7- Δ 24 (ref. 37), which enters through heparan sulfate proteoglycans, were able to kill breast CICs.³⁸ Breast CICs have been identified as a CD44⁺/CD24^{-/low} population in breast carcinoma.^{39,40} These cells represent a small subpopulation and exclusively retain tumorigenicity in the xenograft model. Like brain tumor CICs, breast CICs can be propagated *in vitro* as spheres ("mammospheres") and retain stem cell-like characteristics. CD44⁺/CD24^{-/low} cells freshly sorted from pleural effusions were enriched in a Hoechst 33342 "side population" (from 1% to 7%), a known characteristic of bone marrow stem cells, and expressed the stem cell markers *oct4* and *sox2* (ref. 38). *In vitro*, both Ad5/3- Δ 24 and Ad5.pk7- Δ 24 effectively killed unsorted and CD44⁺/CD24^{-/low} cells compared to an Ad5 wild-type or a replication-deficient control, suggesting tumor tropism and selectivity of these capsid-modified viruses. *In vivo*, no tumor grew in mice injected with Ad5/3- Δ 24-infected CD44⁺/CD24^{-/low} cells. In addition, intratumoral injection of Ad5/3- Δ 24 or Ad5.pk7- Δ 24 into tumors derived from CD44⁺/CD24^{-/low} CICs stopped tumor growth and prolonged animal survival. Bauerschmitz *et al.* from the same group later demonstrated that

tissue-specific promoter (TSP)-controlled Ad5/3 variants also target CD44⁺/CD24⁻ breast CICs.⁴¹ Cyclo-oxygenase-2 (Cox-2), telomerase (hTERT), and multidrug resistance (mdr) protein promoters were used to control E1A expression and showed activated promoter activity in CD44⁺/CD24⁻ cells. *In vitro*, Ad5/3-mdr- Δ 24 was the most oncolytic for CD44⁺/CD24^{-/low} cells freshly collected from two out of three pleural effusions and Ad5/3-hTERT- Δ 35 and Ad5/3-Cox2L- Δ 24 closely followed. *In vivo*, these viruses all showed significant anti-tumor efficacy compared with a mock control against CD44⁺/CD24^{-/low}-derived tumors. Interestingly, in both studies, the proportion of CD44⁺/CD24^{-/low} cells in the recipient tumor returned to the same level as presorted cells [\sim 3% for pleural effusion⁴¹ and \sim 10% for short-term cultured cell line, JIMT-1 (ref. 38)], even when injected with 100% CD44⁺/CD24^{-/low} cells, consistent with asymmetric cell division. After virus treatment, CD44⁺/CD24^{-/low} from JIMT-1 tumors decreased to 1.1% (Ad5.pk7- Δ 24) and \sim 5% (Ad5/3- Δ 24), and the Hoechst side population in CD44⁺/CD24^{-/low} cells decreased from 14% (untreated) to 3%, suggesting the capsid-modified Ads indeed target CICs in the tumors.³⁸ In contrast, the proportion of CD44⁺/CD24^{-/low} cells from fresh pleural-derived tumors remained similar or slightly lower (3.1% vs. 2.6%) after treatment with Ad5/3-mdr- Δ 24 (ref. 41), suggesting perhaps a less effective targeting of the CICs in the primary tumor setting. Although promising results were shown in both studies, complete tumor eradication was not seen in either case. The fact that the viruses efficiently infected CD44⁺/CD24^{-/low} cells *in vitro* indicates a barrier for viruses to reach all tumor cells *in vivo*.

Studies of brain tumor CICs with Ad have also been pursued. Jiang *et al.* showed that tumorspheres derived from surgical GBM specimens express high levels of coxsackie-Ad receptor and the Ad internalization receptors, α v β 3 and α v β 5 integrins, which enabled the Ad mutant Delta-24-RGD to effectively target these cells *in vitro* and prolong survival in tumor-bearing mice.⁴² They further demonstrated that Delta-24-RGD-mediated cell death is via an autophagy process, as indicated by accumulation of autophagic vacuoles and Atg5 and LC3II proteins in the infected cells.

Skog *et al.* first reported using Ad vectors other than the most commonly used serotypes 3 or 5 to target brain CICs.⁴³ Their results showed Ad16p and chimpanzee Ad CV23 effectively target GBM cell lines and both CD133⁺ and CD133⁻ cells freshly isolated from primary brain tumors. In contrast, Ad5 was mainly effective in higher passage established cell lines but very poor in primary specimens, indicating differences between primary and culture-adapted cells relative to Ad5 treatment.

Myxoma virus

Myxoma virus is a member of the poxviridae family, contains a double-stranded DNA genome and causes disease (myxomatosis) in rabbits. Although the virus does not infect normal human cells, it is highly replicative and lytic for many different human cancer cells.⁴⁴ The primary reason for the permissiveness of these cells is their altered signaling pathways, particularly Akt.⁴⁵ In preliminary studies in Paul Beaudry's group at the University of Calgary, Alberta, Canada, Redding *et al.* reported that myxoma virus effectively infected neuroblastoma CIC cultures isolated by Hansford *et al.*⁴⁶ This finding may indicate that neuroblastoma CICs have

altered Akt signaling, which is known to be an important survival signal in neuroblastoma cells,⁴⁷ and suggest this may be an effective virus-tumor combination.

RNA VIRUSES

Reovirus

Reovirus is a double-stranded RNA virus generally considered to be benign in humans, typically causing at most mild respiratory or gastrointestinal ailments. Interestingly, the virus is highly cytopathic for a variety of cultured cancer cell lines and induces rapid tumor regression in mouse xenograft models of a variety of human cancers including those from colon, ovarian, breast, lymphoma, brain and spinal cancer derived cells.⁴⁸⁻⁵³ Reovirus is oncolytic in its naturally isolated state and over the past decade has been in development as a potential cancer therapeutic (Reolysin).⁵⁴ After promising results from phase I/II clinical trials on various cancers, Reolysin is scheduled to begin phase III clinical trials for head and neck cancer in the 4th quarter of 2009.

Recently, we (P.M. and P.W.K.L.) used biopsy cores taken from the primary tumor of a breast cancer patient to grow tumors in the mammary fat pad of immunocompromised mice.⁵⁵ Subsequent reovirus injection induced regression of these patient-derived breast tumors. In this study, breast CICs were identified by the two published methods, tumor cells with CD44⁺/CD24^{-/low} cell surface expression and cells positive for aldehyde dehydrogenase 1 expression (Aldefluor⁺).^{39,56} CIC and non-CIC populations in the reovirus-treated tumors were found to be equally infected and eradicated. As predicted, it appears that characteristics of CICs that make them resistant to common chemotherapeutics and radiation treatments are not hindering factors in reovirus cancer therapy.^{29,57-61}

Reovirus permissiveness has been shown to correlate with the activation status of the Ras signaling pathway(s) in the host cell.⁶² Although Ras mutations are present in only about 30% of all human cancers, the fact that mutations in upstream activators and/or downstream effectors of Ras can also result in enhanced Ras signaling suggests that most cancers are theoretically treatable by reovirus.⁶³ Ras proteins function as molecular switches in many cellular processes including apoptosis, cell cycle transitions, protein translation, cytoskeletal rearrangement, and intracellular vesicle transport.⁶⁴ Although we did not assess Ras activation status, we found that isolated breast CICs and non-CICs had similar levels of total Ras expression.⁵⁵ This finding is congruent with the observation that the two cancer cell subpopulations were equally sensitive to reovirus infection.

The cellular changes undergone by a cancer cell or induced by Ras transformation have favorable effects on multiple steps of reovirus replication.⁶⁵ First, once the virus enters a cell it must undergo an uncoating step in order to initiate the infectious process. This step requires sufficient levels of cathepsin lysosomal proteases that are upregulated in cancer cells and by Ras signaling.⁶⁵⁻⁶⁷ Second, Ras-mediated transformation lends a favorable cellular environment that enhances infectivity of the progeny virus particles, possibly by facilitating post-translational modification of viral proteins, and their subsequent assembly into virions. Finally, cancer cells are significantly more sensitive to reovirus-induced apoptosis at late stages of the infection, resulting in enhanced

release, and therefore spread of the virus through subsequent rounds of infection.^{51,65,68} In this regard, it is interesting to note that JNK activation and NF- κ B have been implicated in reovirus-induced apoptosis and both are altered by Ras-induced transformation and oncogenesis.^{64,69-72} The cumulative effect of these cellular changes results in the heightened sensitivity of cancer cells to reovirus infection, which thus far appears to also manifest in breast CICs. It will be important to determine whether reovirus can also target and kill CICs from other tumor types.

Vesicular stomatitis virus

A member of the rhabdoviridae family, vesicular stomatitis virus (VSV) is a negative sense single-stranded RNA virus that is highly sensitive to the antiviral IFN response. This acute sensitivity to IFN-induced changes effectively blocks infection of the virus in normal cells. It also makes the virus an ideal naturally oncolytic agent as cancer cells often have a deregulated IFN response, allowing for uninhibited VSV replication.⁷³ In addition, tumor cells with defects in Ras, p53 or Myc signaling pathways have been shown to be permissive to VSV.⁷⁴

VSV is an animal pathogen, which can also infect humans. In humans, VSV infections are usually asymptomatic, but it can cause flu-like symptoms, raising some concern about using the virus as a cancer therapeutic in its naturally isolated state. Because the original observations that a laboratory VSV isolate (strain Indiana) efficiently infects and kills tumor cells *in vitro* and *in vivo*,⁷⁵ natural mutants and engineered versions have been isolated with improved oncolytic cancer-killing capabilities and tumor specificity.⁷⁵ For example, the HSV thymidine kinase gene has been incorporated into the VSV genome.⁷⁶ This suicide cassette is designed to enhance killing of VSV-infected tumor cells and also uninfected neighboring tumor cells through the "bystander effect." In another example, interleukin-4 cDNA was incorporated into the VSV genome in order to enhance anti-tumor immunity.⁷⁶ Using an antibody binding domain, Bergman *et al.* engineered a VSV variant that could target Her2/neu overexpressing cells, which is most typical of breast cancers⁷⁷ and plays an important biologic role in breast CICs.⁷⁸ Naturally isolated VSV variants AV1 and AV2 have mutated matrix (M) proteins (responsible for blocking IFN induction in infected cells), and are further attenuated for replication in normal cells but still retain full cancer-killing ability.⁷⁹ This finding led to engineered versions such as recombinant VSV Δ M51, which has reduced toxicity but is highly effective at targeting various primary and metastasized tumors *in vivo*.^{79,80}

Numerous studies have convincingly shown the ability of VSV and the various recombinant strains to induce tumor regression in multiple cancers (*e.g.*, melanoma, lung, colon, brain).⁸¹ In view of increasing evidence of the role of CICs in cancer, the question whether this RNA virus is also able to target and kill CICs remains. Using the VSV recombinant strain, Δ M51, Redding *et al.* are testing the efficacy of VSV to infect and kill the primary human neuroblastoma CICs isolated by Hansford *et al.*²⁵ mentioned previously. Preliminary results suggest these cells may be resistant to VSV infection (N. Redding and P. Beaudry, University of Calgary, personal communication, 5 June 2009). If confirmed, resistance of neuroblastoma CICs to VSV may lead to interesting studies on their biology, including their IFN response.

Table 1 Summary of virotherapy studies using CICs

Cancer type	CIC source	Virus	Result	Comments	Reference
Glioblastoma	P	HSV-1	Mixed	HDACi needed	23
	P	HSV-1	Mixed	ICP34.5 needed	26
	P	HSV-1	Susceptible	CD111 (nectin-1) expression critical	28
	P	Adenovirus	Susceptible	RGD-modified fiber knob	42
	P, CL	Adenovirus	Susceptible	Ad16, Ad CV43	43
Breast	PE	Adenovirus	Susceptible	Capsid-modified Ad5/3 or Ad.pk7	38
	PX	Reovirus	Susceptible	No virus modification or co-therapies	55
	CL	HSV-2	Mixed	HDACi needed	PC
Neuroblastoma	CL	HSV-1	Susceptible	ICP34.5 needed	8
	BM	HSV-1	Mixed	1/3 resistant	U
	BM	Myxoma	Susceptible	Akt dependent?	46
	BM	VSV	Resistant	Reasons not yet known	PC

Abbreviations: BM, bone marrow-derived spheres; CIC, cancer-initiating cell; CL, cell line-derived spheres or cell lines sorted by marker expression; HDACi, histone deacetylase inhibitor; HSV, herpes simplex virus; P, primary tumor-derived spheres; PC, personal communication (see text); PE, pleural effusion sorted by marker expression; PX, primary xenograft, CICs identified by staining; U, unpublished data by some of the authors.

CONCLUDING REMARKS

For many different cancer types there is now considerable evidence supporting the stem cell theory of cancer, which suggests that a relatively small subpopulation of cells (CICs) within a tumor are actually tumorigenic and responsible for generating the bulk of nontumorigenic cancer cells. Although not yet proven, the identification, isolation, and characterization of such cells is likely to be paramount to discover effective new cancer therapies that prevent relapse and improve long-term overall survival. With the recent resurgence of interest in the use of oncolytic viruses as cancer therapeutics, their effects on CICs may ultimately determine whether they play a significant role in improving survival rates. Because they are not subject to the same mechanisms mediating resistance to cytotoxic chemotherapy and irradiation, there is ample reason to postulate oncolytic viruses will be effective at eradicating CICs.

Although this topic is only beginning to be addressed as the identification of CICs in various cancers are revealed, the data so far present a mixed picture. Some CICs appear susceptible to virus infection and some do not, depending on the virus mutation and mechanism of attenuation (Table 1). The disrupted IFN response found in many cancer cells creates an oncolytic virus therapeutic window; virus replication is permitted in cancer cells but is thwarted by normal cells. Some data indicate CICs may have a more robust, intact IFN response than the bulk cancer cells, suggesting this may be a mechanism of relative resistance to virus infection. Thus the selection of genetic mutants that can counteract that response may be critical. Fortunately, histone deacetylase inhibitors also appear to counteract the IFN effects and restore virus permissiveness, suggesting effective treatment with oncolytic viruses in some cases may require such concomitant “virus-enabling” therapies. Although there do not appear to be universal themes yet emerging, of this we are certain: with the myriad different virus types and genetic mutations under investigation as oncolytic agents and the rapidly expanding list of CICs being discovered, the interaction of oncolytic viruses with CICs will be a fruitful area of investigation for years to come.

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