Oncolytic viruses: smart therapeutics for smart cancers

> "OVs are self-replicating biological machines ... creating an army of parasites that spread throughout the tumor."

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Editorial

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In his 2011 American Society of Clinical Oncology (ASCO) Presidential Address, George Sledge (Indiana University Melvin, IN, USA) argued that cancers can be classified as 'stupid or smart' depending upon their mutational load. His thesis was that 'stupid' cancers have a single dominant mutation (e.g., *CML* and *BCR-ABL*) that can be effectively treated by targeting the key oncogenic protein with a single small molecule (e.g., imatinib/Gleevec®, Novartis Oncology, USA). Unfortunately, most patients suffer from 'smart' cancers, which are tumors with multiple 'driver' mutations, each one requiring its own specific inhibitor in order to potentially reverse the malignant phenotype. To improve outcomes for patients infested with smart cancers requires the development of 'smart therapeutics' – treatments that attack cancers in multiple ways. We argue that oncolytic viruses (OVs) are smart therapeutics and, indeed, through genetic engineering can have their 'IQs' raised by several points.

Preying upon activated signaling pathways

OVs are replicating therapeutics that are engineered to grow in tumor cells but are unable to productively infect normal tissues [1,2]. OVs are smarter than small molecules because, rather than trying to inhibit a single oncogenically activated pathway, OVs prey upon entire malignant signaling networks that drive the cancer phenotype. The replication of OVs is strictly dependent upon the malignant activation of cell signaling pathways in tumor cells such that cancer driver mutations are also OV driver mutations. The addiction of OVs to overactive cell signaling means that individual driver mutations in a cancer (e.g., EGF receptor [*EGFR*] and *KRAS*) are not as important to the viral therapeutic as the overall pathway activation state. Lee *et al.* [3] found that, in a lung tumor from a single patient, there were a large number of mutations in the cancer genome, many of these leading to overlapping, redundant activation of the EGFR and parallel pathways. Single molecular targeting of any one of these mutations will not provide therapeutic benefit to the patient. By contrast, clinical OVs, such as vaccinia virus (JX-594, Jennerex Biotherapeutics, CA, USA) or reovirus (Reolysin®, Oncolytics Biotech, Alberta, Canada) require activation of the EGFR pathway to efficiently replicate [1,4]. To the virus, the type or number of activating mutations is irrelevant – as long as there is at least one, the virus will be able to carry out its replicative cycle. Once the virus initiates infection within a tumor cell, it elaborates a series of ingenious viral proteins that usurp the cell's metabolic machinery. The co-opting of transcriptional and translational machinery by the virus means that the infected cell will be unable to support its own basic functions and succumb, liberating OV particles that can move on to the next tumor cell.

Smart cancers are not altruistic

Tumor cells, in their quest for immortality, have found ways to overcome or attenuate their apoptotic programs. Normal cells have functional apoptotic programs with multiple roles but one important use of this pathway is to block virus spreading during the course of an infection. In this respect, normal cells are 'altruistic', preferring to 'commit suicide' following infection in order to blunt virus growth and protect their neighbors. Many pathogenic viruses encode inhibitors of apoptosis, allowing them to productively infect normal tissues and circumvent cellular antiviral programs. OVs are engineered to have mutations in antiapoptotic functions and, thus have a limited capacity to productively infect normal tissues. Cancer cells

Keywords

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are not altruistic and, since they have eliminated their apoptotic programs, are powerless to resist OV infection and spread.

OV tumor targeting & self-amplifying dosing

One challenge to the treatment of metastatic cancer is delivery of an optimal therapeutic drug dose to all the sites of disease within the patient. Large concentrations of the drug must be delivered intravenously to reach the tumor bed, but, even then, high interstitial pressure or poor tumor perfusion can prevent drugs from reaching their intended target [5]. For most drugs there are no mechanisms of selective delivery and so the patient must be effectively saturated with the therapeutic to achieve beneficial concentrations within the tumor.

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An OV can be engineered to specifically recognize either the tumor cell surface [6] or the tumor vascular endothelium [7], facilitating selective delivery. For many OVs in clinical development, targeting is not at the cell surface but rather determined by malignantly activated intracellular signaling pathways [1,8]. Regardless of the mode of delivery to the tumor bed, because OVs are self-replicating biological machines, they can copy their genetic information, express virally encoded proteins and self-assemble, generating more therapeutic viral particles *in situ*. In principle, only a handful of OV particles needs to infect a tumor for self-amplification to occur, creating an army of viral parasites that spread throughout the tumor. In reality, clinical data suggest that as many as several thousand viral particles need to seed a tumor before the OV can successfully spread [9]. Many of the barriers to drug entry into the tumor exist for virus particles; however, some innate OV characteristics and other traits that can be engineered into the virus can lead to more effective therapeutic spread. Some OVs spread via cell–cell contact and/or fusion, thus mitigating interstitial pressure concerns. For highly fibrotic cancers, where tumor nests are encased in extracellular matrix, new OVs are being developed that encode proteases, allowing the virus to burrow between tumor beds [10,11].

OVs have learned to navigate the perils of the bloodstream

Barriers to the delivery of therapeutic viruses to metastatic tumors via the vascular system include immune cells, antibodies, complements, and a variety of scavenger cells that line the endothelium of vessels in the liver and spleen. Viruses have evolved strategies to overcome many of these barriers. For instance, viruses have turned the tables on their mammalian hosts and 'ride or hitchhike' on the very immune cells meant to target them in order to gain carriage into tumor beds [12,13]. Sometimes, the hitchhiking virus is protected from neutralizing antibodies in the circulation by this cell association. Some OVs encode inhibitors of the complement system within their genomes to overcome this barrier [14]. Poxviruses not only encode complement inhibitors, but also create multiple viral isoforms, including a 'cloaked or stealth' version that can avoid antibody neutralization and enhance virus spread [14]. The demonstration of dose-dependent delivery of OVs to tumors in cancer patients after intravenous administration demonstrates that at least some of the barriers found within the bloodstream can be overcome simply by using 'saturating' doses [9].

Targeting tumor vasculature

Any clever General knows that a simultaneous direct frontal assault is more effective when the enemy's supply lines have been cut off. OVs use this strategy to aid in the attack upon their tumor foes. OVs, either through genetic engineering [15] or as part of their natural biology [16], have the capacity to specifically infect and destroy tumor blood vessels. Engineered viruses have been created that simultaneously recognize the surface antigens of tumor vessels and tumor cell receptors. This affords them the opportunity to productively infect and destroy the vascular pipeline that feeds the cancer, while also wreaking havoc on the tumor by direct infection [17]. Many natural viruses have evolved mechanisms to infect endothelial cells as part of their natural pathogenic program. Some of these viruses can be selected or engineered to become oncolytic, and, thus have the ability to infect both their ultimate target, the tumor and the tumor's associated blood vessels. In these cases, the selectivity for tumor vasculature is not at the cell surface but rather it exploits signaling pathways that are malignantly activated in the tumor microenvironment. In experimental mouse models, the infection of tumor vasculature leads to the specific formation of microclots

that, remarkably, are completely restricted to the malignancy [16,18]. These intravascular clots cause a catastrophic loss of blood flow into the tumor and initiate massive cancer cell death, even in uninfected areas. Therefore, while OVs directly infect and kill tumor cells, they also use this second strategy to kill throughout the tumor bed. The genius of this approach is that even if the tumor is somewhat refractory to OV infection, destruction of its supporting vasculature could still lead to good therapeutic outcomes.

Smart viruses expose stealth tumors to the immune system

In their recent update of the 'Hallmarks of cancer', Hannahan and Weinberg include immune evasion as a key property of the successful malignancy [19]. While smart tumors mutate multiple signaling molecules/pathways to gain a growth advantage, in doing so, they may also generate a vast array of new tumor antigens that can be potentially recognized by the patient's immune system. It is now clear that smart tumors adapt a variety of different strategies to hide from immune surveillance programs, which are active in all of us. Tumors downregulate self-surface MHC molecules, secrete immunosuppressive cytokines to paralyze immune effector cells and dampen innate immune responses that are incompatible with rapid and unlimited cell growth. When OVs initiate tumor-specific infections, they trigger localized inflammatory reactions [18]. This inflammatory cascade uncloaks the stealth tumor and leads the patient's immune response right to the offending tumor bed. In animal models, there is no doubt that activation of antitumor immune responses is an important component of the therapeutic benefit of OVs [20]. This is also likely to be true of clinically relevant OVs, such as JX-594 [21], and the Amgen (CA, USA) product OncoVEX, which is being coined an oncovaccine. The antitumor immune responses triggered during oncolysis by the OncoVEX product are truly remarkable and lead to durable responses in a significant portion of melanoma patients [22]. In general, it is felt that immune responses to viruses are likely to curb the effectiveness of OV therapeutics. Given that smart tumors create a zone of immune suppression in their microenvironment, they may be playing into the hands of OVs by providing them with an immune sanctuary where the virus can also remain, at least transiently, invisible to the patient's immune system.

What is good for the tumor is good for the OV

Herein, we have provided examples of how the mutated pathways that promote tumor growth also create an environment that favors OV replication. OVs are easy to engineer and, as we learn more about how cancers evolve and become genetically unique from their normal counterparts, it will be possible to further enhance OVs, allowing them to become formidable smart therapeutics. OVs have the potential to become effective anticancer therapeutics and certainly the clinical data to date suggest that they may be amongst the safest therapeutics currently in clinical use and/or testing.

Financial & competing interests disclosure

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