Replicating poxviruses for human cancer therapy

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(Received Jan 21, 2015 / Revised Mar 4, 2015 / Accepted Mar 19, 2015)

Naturally occurring oncolytic viruses are live, replication-proficient viruses that specifically infect human cancer cells while sparing normal cell counterparts. Since the eradication of smallpox in the 1970s with the aid of vaccinia viruses, the vaccinia viruses and other genera of poxviruses have shown various degrees of safety and efficacy in pre-clinical or clinical application for human anti-cancer therapeutics. Furthermore, we have recently discovered that cellular tumor suppressor genes are important in determining poxviral oncolytic tropism. Since carcinogenesis is a multi-step process involving accumulation of both oncogene and tumor suppressor gene abnormalities, it is interesting that poxvirus can exploit abnormal cellular tumor suppressor signaling for its oncolytic specificity and efficacy. Many tumor suppressor genes such as p53, ATM, and RB are known to play important roles in genomic fidelity/maintenance. Thus, tumor suppressor gene abnormality could affect host genomic integrity and likely disrupt intact antiviral networks due to accumulation of genetic defects, which would in turn result in oncolytic virus susceptibility. This review outlines the characteristics of oncolytic poxvirus strains, including vaccinia, myxoma, and squirrelpox virus, recent progress in elucidating the molecular connection between oncogene/tumor suppressor gene abnormalities and poxviral oncolytic tropism, and the associated preclinical/clinical implications. I would also like to propose future directions in the utility of poxviruses for oncolytic virotherapy.

Keywords: oncolytic virus, poxvirus, vaccinia virus, myxoma virus, squirrelpox virus, oncogenes, tumor suppressor genes

Introduction

Oncolytic viruses are live, replication-proficient viruses that preferentially infect human cancer cells while sparing normal cell counterparts. Such replication-proficient viruses provide a series of potentially viable anti-cancer therapeutic approaches. Oncolytic viruses have many advantages over the use of conventional chemotherapy/radiotherapy or replication-incompetent viral vectors. First, they generally target cancer cells specifically, because of their natural or engineered reduced ability to replicate in normal cells, while replicating vigorously in and killing transformed cells. Second, as compared to replication-incompetent viral vectors, they can propagate from initially infected cancer cells to surrounding or distant cancer cells, thereby achieving a wide distribution and exerting potent anti-cancer effects (Parato et al., 2005; Hartkopf et al., 2011; Russell et al., 2012).

Because of these unique features of the replicating nature of oncolytic viruses, they are highly dependent on the host cell physiology for optimal performance as viral cancer-targeting agents. Many naturally occurring viruses have shown great potential as cancer-targeting agents by exploiting various oncogene signaling pathways that are established by host cancer cells during tumorogenesis (Strong et al., 1998; Roberts et al., 2006; Wang et al., 2006). However, carcinogenesis is a multi-step process involving accumulation of not only oncogene abnormalities but also tumor suppressor gene abnormalities, and we recently discovered that cellular tumor suppressor genes such as p53, ATM (Ataxia telangiectasia mutated), and RB (Retinoblastoma-associated) are also important in determining oncolytic viral tropism, including in poxvirus (Kim et al., 2010). Thus, an important mechanism of viral oncolysis can be established by both cellular oncogene and tumor suppressor gene abnormalities.

Since the eradication of smallpox in the 1970s with the aid of vaccinia viruses, vaccinia viruses and other poxvirus genera have shown various degrees of safety and efficacy in pre-clinical or clinical application for human anti-cancer therapeutics. Here, I review recent progress in molecular studies and preclinical/clinical aspects of replication-proficient poxvirus oncolysis.

Origin of oncolytic poxviruses

Poxviruses, which belong to the Poxviridae family, are ubiquitous, enveloped viruses that replicate entirely in the cytoplasm of vertebrate or invertebrate cells. Poxvirus particles (virions) can be externally enveloped virion (EEV), although the intracellular mature virion (IMV) form of the virus, which contains a different envelope, is also infectious. They vary in shape depending upon the species, but are generally brick- or oval-shaped (similar to a rounded brick) wrapped by the endoplasmic reticulum. The virion is exceptionally large at

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around 200 nm in diameter and 300 nm in length, and carries its genome in a single, linear, double-stranded segment of a DNA molecule comprising 130 to 300 kb pairs (Moss, 2013).

Vaccinia virus is a member of the Orthopoxvirus genus of the Poxviridae and is the most intensively studied poxvirus. It is most well known as the live vaccine virus that was used to eradicate smallpox caused by the variola virus, a feat completed in the 1970s and that remains the greatest triumph for the World Health Organization to date (Ellner, 1998). Yet, despite the effectiveness of vaccinia virus in eradicating smallpox, its origin and natural history are unknown and remain an enigma of virology (Baxby, 1977; Wilkinson, 1982). The live vaccinia Lister strain was developed at the Lister Institute in the United Kingdom. From 1968 to 1971, the Lister strain became the most widely used vaccine throughout the world (Rosenthal et al., 2001). More recently, the oncolytic nature of the Lister strain has been studied by several research groups (Timiryasova et al., 1999; Chen et al., 2001; Hung et al., 2007). Currently, a modified version of the Lister strain is in clinical trials for treating various human cancers as well as for feline/canine cancer therapy (Gentschey et al., 2014; Mell et al., 2014). The live vaccinia Wyeth strain was one of the smallpox vaccine viruses used mainly in the Americas and West Africa during the worldwide vaccination campaign (Jacobs et al., 2009; Nalca and Zumbrun, 2010). The oncolytic nature of the Wyeth strain has also been widely studied (Mastrangelo et al., 1999; Liu et al., 2014), and a modified version of the Wyeth strain is in clinical trials for treating various human cancers (Mastrangelo et al., 1999; Park et al., 2008; Heo et al., 2013). The live vaccinia Western Reserve (WR) strain was derived from serial passaging the New York City Board of Health (NYCBH) strain in the mouse brain, and has been shown to replicate to high titers in various mouse organs (Kaplan, 1989; Brandt and Jacob, 2001; Brandt et al., 2005). The oncolytic nature of the WR strain has been studied (Gnant et al., 1999; McCart et al., 2001; Thorne et al., 2007; Autio et al., 2014; Parviainen et al., 2015), and a modified version is in a clinical trial for treating various human cancers (Mastrangelo et al., 1999; Park et al., 2008; Heo et al., 2013). The live vaccinia Ankara (MVA) strain was derived in the late 1950s by passaging the chorioallantois VACV Ankara (CVA) strain of vaccinia virus more than 570 times in chick embryo fibroblast cells, resulting in a host range-restricted virus that is replication-defective in most mammalian cells (McCurdy et al., 2004). This highly attenuated strain is unable to fully replicate in human cells and presented no adverse reactions in clinical trials (Sutter and Moss, 1992). MVA was safely used to vaccinate over 100,000 people in Germany (Mayr, 2003), yet its effectiveness against smallpox remains untested. Due to its viral replication potential being severely compromised, MVA has been used as a nonreplicating anti-cancer vector to deliver various transgenes rather than for replicating oncolytic virotherapy (Sutter and Moss, 1992; Carroll et al., 1997; Drexler et al., 1999). Currently, a modified version of the MVA strain is in clinical trials for treating various human cancers (Larocca Schlom, 2011; Amato et al., 2012; Gómez et al., 2013). Strain LC16m8 was developed in Japan in 1975, by passaging the Lister strain through primary rabbit kidney epithelial cells (PRK) at a low temperature (30°C) (Kenner et al., 2006). The Lister virus was initially passaged 36 times through PRK cells, and individual clones were then evaluated for growth on monkey kidney Vero cells, in order to evaluate their ability to replicate in primate tissues. Strain LC16, which grew to the lowest titer in Vero cells, was passaged 6 more times under identical conditions. Eventually, LC16mO, which formed medium-sized pocks on chick chorioallantoic membranes (CAM), was isolated from this stock and passaged 3 more times on PRK cells. LC16m8 was then isolated from this latter stock as a clone that both replicated poorly in Vero cells and formed small plaques on CAM, PRK, and continuous rabbit kidney epithelial RK13 cells. Currently, modified versions of LC16m series strains (LC16mO, LC16m8, etc.) are in preclinical studies for potential treatment of various human cancers (Hikichi et al., 2011). Raccoonpox virus (RCNV) is also a member of the Orthopoxvirus genus and is closely related to the vaccinia and cowpox viruses. RCNV was first reported in 1964 by Herman, and was isolated from a naturally occurring poxvirus from the respiratory tract of raccoons inhabiting an undeveloped forest and swamp area near Aberdeen, Maryland (Herman, 1964; Thomas et al., 1975). Recently, a modified version of the RCNV strain is in preclinical studies for its potential in treating various human cancers (Evgin et al., 2010).

Yaba-like disease virus (YLDV) is a member of the Yatapoxvirus genus of the Poxviridae family. YLDV is closely related to Yaba monkey tumor virus (YMTV) and tanapoxvirus, which also belong to the Yatapoxvirus genus. YLDV was first recognized in 1965 and 1966 in monkey caretakers who were working at primate centers in the United States, and was traced to a single source (Esparza, 1971). YLDV infection in the caretakers produced a brief fever and the development of a few firm, elevated, round, necrotic maculopapular nodules, followed by complete resolution of the infection. Recently, a modified version of the YLDV strain is in a preclinical study for human ovarian cancer therapy (Hu et al., 2001).

Myxoma virus (MYXV) is a member of the Leporipoxvirus genus of the Poxviridae family. Two distinct types of MYXV have been identified: South American MYXV (Lausanne strain; Brazil/Campinas/1949/1), which circulates in the tapeti (Sylvilagus brasiliensis), and Californian MYXV, which circulates in the brush rabbit (Sylvilagus bachmani). Each virus is highly adapted to its host, causing a benign cutaneous fibroma at the site of inoculation. Both types of MYXV infect the European rabbit (Oryctolagus cuniculus), causing myxomatosis with mortality rates reaching up to virtually 100% (Fenner, 1983; Kerr et al., 2012). The Lausanne strain of MYXV originated from a Brazilian rabbit, but is so-named because it was obtained from a laboratory in Lausanne, Switzerland (Regnery, 1971). Currently, a modified version of the MYXV Lausanne strain is in preclinical studies for treating various human cancers (Lun et al., 2005; Kim et al., 2009, 2010; Kim and Johnston, 2014; Zemp et al., 2014).

Squirrelpoxvirus (SQPV) is a member of an as-yet-unassigned genus of the Poxviridae family. SQPV was isolated from a grey squirrel (Sciurus carolinensis) in Maryland in 1953 and initially placed into the genus Leporipoxivirus by Kilham et al. (1953); it was later thought to be a member of the genus Parapoxvirus (Housawi et al., 1998), but a sub-