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The mechanisms of genetically modified vaccinia viruses for the treatment of cancer

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Abstract

The use of oncolytic viruses for the treatment of cancer is an emerging field of cancer research and therapy. Oncolytic viruses are designed to induce tumor specific immunity while replicating selectively within cancer cells to cause lysis of the tumor cells.

While there are several forms of oncolytic viruses, the use of vaccinia viruses for oncolysis may be more beneficial than other forms of oncolytic viruses. For example, vaccinia viruses have been shown to exert their anti-tumor effects through genetic engineering strategies which enhance their therapeutic efficacy. This paper will address some of the most common forms of genetically modified vaccinia viruses and will explore the mechanisms whereby they selectively target, enter and destroy cancer cells. Furthermore, this review will highlight how vaccinia viruses activate host immune responses against cancer cells and will address clinical trials evaluating the tumor-directed and killing efficacy of these viruses against solid tumors.

Keywords: Virotherapy; Oncolytic vaccinia virus; Cancer; Orthopoxvirus

1. Introduction

In the early twentieth century, clinicians introduced the radical concept that viruses may be used to treat cancer [1,2]. Although revolutionary, the notion died down due to concerns from side effects and the lack of substantial findings

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[1]. Termed virotherapy, it wasn't until the late 1990s that clinicians became re-interested in the use of viruses to target and treat cancer [1]. Modern technology as well as the introduction of gene therapy provided new enlightenment. As a result, the use of oncolytic viruses for the treatment of cancer has now become an emerging field of cancer research and treatment. Of the viruses currently under investigation, the Oncolytic Vaccinia Virus (VACV) has been one of the most extensively studied. The vaccinia virus belongs to the poxviridae family, which are large, double stranded DNA viruses closely related to cowpox and monkeypox. Infection with VACV is characterized by the formation of pock lesions on skin [3]. With a genome the size of ∼190 kbp, VACV is considered to be a large and complex animal virus and consists of many strains of which the most prominent include: Lister, Wyeth, and Western Reserve. With regard to the treatment of cancer, oncolytic VACV has been shown to replicate and lyse tumor cells within 72 h post-infection [4]. It has also been shown to exhibit broad tumor tropism and can move through the bloodstream to target distant tumors [4]. Importantly, recombinant oncolytic VACV, which has been genetically modified, selectively targets tumor cells while sparing non-malignant cells, making it an ideal agent as it minimizes damage to healthy tissues [5]. Non-genetically modified VACV indiscriminately targets both tumor cells and healthy cells. Furthermore, VACV has been reported to replicate in the cytoplasm of cells, preventing integration of viral DNA into host chromosomes and thus passage of viral progeny to daughter cells [6]. These features of VACV have made it an ideal agent for the treatment of cancer.

There are four infectious forms of VACV produced during the virus's life cycle [7–9]. These forms include: Intracellular Mature Virion (MV), Intracellular Enveloped Virion (IEV), Cell-associated Enveloped Virion (CEV) and Extracellular Enveloped Virion (EEV). Out of the four infectious forms, MV and EEV are the most common produced during assembly. Assembly takes place in cytoplasmic factories and involves the usage of non-infectious precursors called "crescents" [8].

The MV has been suggested to be the most abundant form of VACV, a feature which may be related to its early assembly during maturation [6]. Consisting of a single lipid bilayer, the MV is the simplest form of VACV and as such, is principally used in research [6]. Sometimes MV will morph into an EEV, a process that is accomplished as a result of the MV exiting cytoplasmic crescents via microtubules to undergo additional modifications [8]. These new modifications include the assembly of an additional membrane, which is formed by viral transport through endosomal or trans-golgi cisternae [6]. While EEV's outer envelope is unstable *ex-vivo*, it is able to spread more rapidly than MV due to its early release from cells following viral replication [10]. Furthermore, EEV is unique from MV in that it has fewer viral antigens exposed on its outer surface and additionally incorporates host cell proteins, enabling it to go undetected by the host's immune system [11]. This feature thus limits its destruction prior to its arrival at the tumor [11]. Importantly, VACVs, including MV and EEV, are able to accommodate multiple large transgenes [4] improving selective tumor targeting and killing. In this manner, the mechanisms surrounding the endogenous features of VACV in addition to genetic modifications enabling VACV tumor entry and tumor cell death will be discussed in this paper. It is anticipated that this information will give light to new mechanisms whereby VACVs may be used as a frontline form of chemotherapy in the future.

2. VACV targeting of tumor cells

The targeting of tumor cells while sparing normal, healthy cells is crucial when it comes to finding a treatment for cancer. Since several wild-type VACVs have been reported to possess inherent affinity to tumor cells [12], genetic modifications have been introduced into VACVs to further improve selective tumor cell infection and death and/or viral replication. The most common form of genetically modified oncolytic VACV is VVdd, a double-deletion mutant [13].

VVdd, originating from the Western Reserve strain, is an attenuated VACV that offers the same efficiency in destroying tumors as the wild-type VACV while concomitantly allowing selective targeting of tumor cells due to its deleted open reading frames (ORFs): Vaccinia growth factor (VGF) and thymidine kinase (TK) [13]. VGF, an extracellular viral growth factor, has been shown to prime neighboring, healthy cells for VACV infection [13,14]. As a result, when VGF is deleted, viral stimulation of neighboring cells will not occur, sparing uninfected, healthy cells [13,14]. TK, responsible for phosphorylation in the pyrimidine salvage pathway, has been reported to enhance viral replication in non-dividing cells [13]. When TK is deleted, viral replication will be restricted to rapidly dividing cancer cells in the G2 and S phases of the cell cycle thus sparing normal cells. [31]. Overall, deletion of TK and VGF synergistically lead to a VACV that selectively targets tumor cells without decreasing its pathogenicity [13].

Although VVdd showed great potential with regard to tumor targeting, researchers have sought to engineer more sophisticated recombinants to enable potent tumor targeting and destruction. It was determined that post-transcriptional regulation of genes is a necessary factor for viral-specific targeting of tumor cells [15]. As a result, a recombinant VACV, referred to as microRNA (miRNA) regulated vaccinia virus (MRVV), was derived from the Lister strain and was engineered to alter the expression of B5R, a gene found in EEV which is responsible for viral morphogenesis, trafficking, and dissemination and contributes to non-specific targeting of both tumor cells and healthy cells [15]. In this study, Hikichi et al. [15] examined the effects of the miRNA let-7a (miRlet-7a) on tumor-specific targeting and replication. miRlet-7a is ubiquitously expressed in normal, healthy cells where it regulates development, cell differentiation and apoptosis and acts as a tumor suppressor [16,17]. In most tumor cells, miRlet-7a is down-regulated which leads to