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Antibody neutralization of retargeted measles viruses

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ABSTRACT

The measles virus (MV) vaccine lineage is a promising oncolytic but prior exposure to the measles vaccine or wild-type MV strains limits treatment utility due to the presence of anti-measles antibodies. MV entry can be redirected by displaying a polypeptide ligand on the Hemagglutinin (H) C-terminus. We hypothesized that retargeted MV would escape neutralization by monoclonal antibodies (mAbs) recognizing the H receptor-binding surface and be less susceptible to neutralization by human antisera. Using chimeric H proteins, with and without mutations that ablate MV receptor binding, we show that retargeted MVs escape mAbs that target the H receptor-binding surface by virtue of mutations that ablate infection via SLAM and CD46. However, C-terminally displayed domains do not mediate virus entry in the presence of human antibodies that bind to the underlying H domain. In conclusion, utility of retargeted oncolytic measles viruses does not extend to evasion of human serum neutralization.

Introduction

Oncolytic virotherapy is an emerging treatment modality for cancer, which exploits viruses that preferentially infect and kill cancer cells. These oncolytic viruses include naturally occurring viruses and viruses that have been engineered for tumor selectivity (Eager and Nemunaitis, 2011; Donnelly et al., 2012; Russell et al., 2012). Oncolytic measles virus (MV) vaccine strains, in particular a laboratory adapted strain of Edmonston vaccine lineage (MV-Edm), has demonstrated therapeutic potential against different solid tumors and hematologic malignancies such as hepatocellular carcinoma (Blechacz et al., 2006), breast cancer (McDonald et al., 2006; Iankov et al., 2010), prostate cancer (Msaouel et al., 2009a, 2009b), ovarian cancer (Peng et al., 2002; Hasegawa et al., 2006), multiple myeloma (Peng et al., 2001; Dingli et al., 2004), lymphoma (Grote et al., 2001) and glioblastoma multiforme (Phuong et al., 2003) in preclinical studies. MV-Edm is also being tested clinically for the treatment of multiple myeloma (NCT00450814), ovarian cancer (Galanis et al., 2010; NCT00408590), glioblastoma multiforme (US-0770) and mesothelioma (NCT01503177).

MV is an enveloped, negative-strand RNA virus of the family *Paramyxoviridae* (Griffin, 2007). MV-Edm has a tropism for three cellular receptors: the signaling lymphocyte activating molecule (SLAM), expressed on activated T and B cells and macrophages (Tatsuo et al., 2000; Hahm et al., 2003; Schneider-Schaulies et al., 2002a, 2002b); Nectin-4, a cellular adhesion molecule expressed in the placenta, trachea, oral mucosa, nasopharynx, and lungs (Reymond et al., 2001; Noyce et al., 2011) and over-expressed on several types of cancer (Derycke et al., 2010; Takano et al., 2009; Fabre-Lafay et al., 2005) and CD46 which is a cellular receptor for laboratory-adapted MV strains (Naniche et al., 1993). CD46 is a regulator of complement activation (Naniche et al., 1993; Dorig et al., 1993) that is ubiquitously expressed on all human nucleated cells and over-expressed on many different cancer cell types making them highly susceptible to MV-Edm infection and its cytopathic effects (Anderson et al., 2004).

MV-Edm can be retargeted to specific tumor cells by linking a single-chain antibody (single chain fragment variable, scFv) or naturally occurring ligand to the virus attachment hemagglutinin (H) glycoprotein displayed on the virus surface. The ablation of receptor CD46 and SLAM binding sites limits virus attachment and entry to cells expressing the receptor for the scFv or ligand linked to H. Retargeted MV-Edm derivatives retain their oncolytic activity against xenografts expressing target receptors (Nakamura et al., 2005; Allen et al., 2006, 2008; Paraskevakou et al., 2007; Hasegawa et al., 2006; Jing et al., 2009; Hummel et al., 2009;

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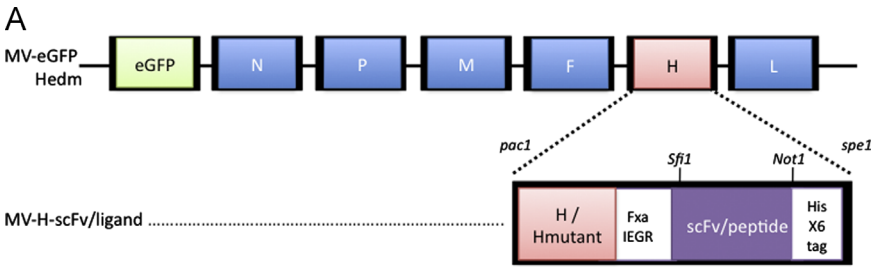
Ungerechts et al., 2007; Yaiw et al., 2011). A variety of scFv's have been displayed on H against different receptors: epidermal growth factor receptor (EGFR) (Nakamura et al., 2005; Paraskevakou et al., 2007); EGFRvIII (Nakamura et al., 2005; Allen et al., 2006); HER2/neu (HER2: Human Epidermal Growth Factor Receptor 2) (Hasegawa et al., 2007), CD20 (Ungerechts et al., 2007; Yaiw et al., 2011); folate receptor alpha (Hasegawa et al., 2006); CD38 (Nakamura et al., 2005); carcinoembryonic antigen (CEA) (Ungerechts et al., 2007), prostate-specific membrane antigen (PSMA) (Liu et al., 2009) and an unidentified receptor over-expressed on multiple myeloma cells that can be targeted by Wue scFv (Hummel et al., 2009). Ligands linked to H have also successfully redirected entry, for example: amino-terminal fragment of urokinase plasminogen activator (uPA) targeting uPA receptor on breast tumors and tumor stroma (Jing et al., 2009); snake venom peptide echistatin, targeting integrins αvβ3 and α5β1 expressed on vascular endothelium (Hallak et al., 2005); single-chain T-cell receptor (scTCR) targeting a specific peptide/MHC complex (Peng et al., 2004) and interleukin-13 targeting gliomas (Allen et al., 2008).

One of the major hurdles for oncolytic virotherapy is preexisting immunity against the oncolytic virus (Parato et al., 2009; Willmon et al., 2009). Measles oncolytic virotherapy is limited by preexisting immunity due to wide-spread global vaccination against measles (Russell and Peng, 2009). The hemagglutinin attachment protein is the major target for neutralizing antibodies (Bouche et al., 2002) that tend to cluster at the receptor binding surface targeting a conserved neutralizing antigenic region (Hashiguchi et al., 2007; Hashiguchi et al., 2011b; Santiago et al., 2010; Ertl et al., 2003; Tahara et al., 2013a). Retargeted MV derivatives have two modifications that could potentially destroy or shield epitopes within the receptor-binding surface. The first modification is a set of two (Y481A and R533A) or four (Y481A, R533A, S548L and F549S) mutations that ablate infection via CD46 and SLAM (Nakamura et al., 2005). The second modification is the scFv or ligand linked to the H C-terminus used to retarget MV to specific receptors. This additional polypeptide domain could shield one or more antibody epitopes and protect the virus from neutralization (Kneissl et al., 2012). Should the utility of retargeted oncolytic MVs extend to evasion of serum neutralization it would render them superior to MV derivatives currently tested clinically.

In this study we used chimeric H proteins with and without mutations that ablate MV receptor binding to determine if these mutations protect MV-Edm from mAbs targeting the mutated receptor-binding surface. We investigated if the displayed domain can shield mAb epitope(s) and if the size of the domain determines how well an epitope is protected. We then addressed the question if retargeted MV derivatives evade human serum



B

Virus name	Mutations in H	scFv/ligand (size, amino acids)	Tropism for Vero-His cells via Receptors:
MV-Hedm			CD46
MV-H_{AALS}	Y481A, R533A, S548L, F549S	His(6)	αHis
MV-H-Ech		Echistatin (49), His(6)	CD46, αHis
MV-H_{AALS}-Ech	Y481A, R533A, S548L, F549S	Echistatin (49), His(6)	αHis
MV-H-Wue.scFv		Wue scFv (245), His(6)	CD46, αHis
MV-H_{AALS}-Wue.scFv	Y481A, R533A, S548L, F549S	Wue scFv (245), His(6)	αHis
MV-H_{AALS}-EGFR.scFv	Y481A, R533A, S548L, F549S	EGFR scFv (245), His(6)	αHis, EGFR
MV-H82-EGFR.scFv	L284S, E395K, E398G, E535N, H536A, A537T, Y310C, D416N, S546N, R547A, S550T, F552N, Y553G, P554T, S590N, G592S	EGFR scFv (245), His(6)	CD46 ?, αHis, EGFR

Fig. 1. Recombinant oncolytic measles viruses used in this study. (A) Schematic representation of MV.eGFP-edm (edmonston vaccine lineage, edm). The gene encoding enhanced green fluorescent protein (eGFP) is at position 1 followed by genes coding for N: nucleoprotein, P: phosphoprotein, M: matrix, F: fusion protein, H: hemagglutinin and L: large protein. Retargeted viruses are generated by replacing the H gene (Hedm) in MV-eGFP with retargeted H using pac1/spe1 restriction enzymes. Retargeted H can be either CD46/SLAM tropic (H) or CD46/SLAM blind due to mutations Y481A, R544A, S548L, and F549S (H_{AALS}). Retargeted H or H_{AALS} has a C-terminal scFv/ligand that can be exchanged with restriction enzymes sfi1 and Not1. A 6-histidine peptide (Hisx6) is attached to the C-terminus of scFv/ligand or H_{AALS}. The Hisx6 acts as a ligand for entry and propagation in Vero cells engineered to express anti-Hisx6 scFv (Vero-HIS cells). The factor Xa (IEGR) cleavage site allows for the removal of the scFv/ligand/Hisx6 from H and is used to demonstrate that a blind retargeted MV infects cells via scFv/ligand or Hisx6. (B) The table lists the recombinant viruses used in this study; the mutations in H; the scFv or ligand displayed on H and its size in amino acids. All viruses encode eGFP. For simplicity MV-eGFP is referred to as MV-Hedm. MV-H82-EGFRscFv has a H glycoprotein with 16 mutations designed to protect seven epitopes from monoclonal antibodies used in this study and is used as a control.