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Engineered measles virus Edmonston strain used as a novel oncolytic viral system against human neuroblastoma through a CD46 and nectin 4-independent pathway ☆

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ABSTRACT

Neuroblastoma (NB) is the most common extracranial solid tumor in children. In this study, we investigated the potential antitumor capability of the engineered Edmonston strain of the carcinoembryonic antigen-expressing measles virus (MV-CEA) against human NB. The infection of a variety of NB cell lines, including SK-N-SH, SMS-KCNR, and primary NB cells, resulted in significant cytopathic effects. None of the NB cell lines showed an overexpression of the measles virus receptor CD46 and nectin 4, but the cell lines did support robust viral replication. The efficacy of this approach was examined in murine SK-N-SH xenograft models. Flow cytometry and TUNEL assays indicated an apoptotic mechanism of cell death. In summary, MV-CEA has potent therapeutic efficacy against NB mediated by a CD46- and nectin 4-independent pathway.

1. Introduction

Neuroblastoma (NB) is the most common extracranial solid tumor in children, accounting for up to 10% of the incidence of pediatric tumor malignancies [1,2]. NB is a typical type of tumor found in developing tissues, being diagnosed primarily during early childhood. Worldwide, NB is the third most common cause of cancer mortality in pediatric patients, causing a large number of deaths annually. The National Cancer Institute reported that the incidence of NB was 10.2/100,000 during the period 1975–2000 [3].

NB is a tumor of the sympathetic nervous system and is primarily located along the migration path of the neural-crest-derived cells. Significant effort has been devoted to the search for the molecular markers of NB, which has led to some advances in high-risk group staging and the identification of pathways for targeted therapies. The biological heterogeneity of NB correlates with a wide spectrum of clinical entities. Some NB cases spontaneously regress, whereas metastatic NB cases have a dismal prognosis [4]. High-risk cases of NB, such as children with NB metastasis to distant sites (e.g., lymph nodes, bone, bone marrow, liver and skin), are a major challenge for pediatric oncologists. Despite a variety of therapeutic options that include surgery, chemotherapy and local therapies, the prognosis for metastatic NB remains poor. However, many therapeutic alternatives are being explored including local therapy, immunotherapy and gene therapy [5–7]. The current approach of gene therapy for metastatic NB recommends viral and nonviral gene therapy systems. Unfortunately, most of these systems suffer from serious drawbacks such as low efficacy and safety risks [8]. The growing incidence, the lack of effective therapies and the devastating prognosis of metastatic NB have greatly increased the urgency for new therapeutic agents that are both safe and effective.

Oncolytic therapy has limited side effects and uses replication-competent viruses that replicate in the tumor cells and kill the cells lytically. This therapy has shown great potential in the treatment of multiple tumors such as lymphoma, ovarian cancer, mesothelioma, breast cancer, and renal and hepatocellular carcinoma [9–15]. Oncolytic therapy has also been proven effective as a human NB therapy [16–22]. Among the numerous oncolytic virus systems, the attenuated Edmonston vaccine strain of the measles virus (MV-Edm) has been proven safe and effective when genetically modified [23–27]. It exerts its cytopathic effect by fusing infected cells with

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the surrounding cells, forming multinucleated syncytia. This event is followed by cell death through apoptotic or nonapoptotic mechanisms [24,26,28].

The measles virus (MV) enters the cells through the interaction of the H-glycoprotein with the MV receptors, CD150 (signaling lymphocyte-activation molecule, SLAM) and CD46 [29–32]. Of note, the wild-type measles virus enters more efficiently through the SLAM receptor, whereas the Edmonston vaccine strain of measles virus enters the cells predominantly through the CD46 receptor [30–32]. The MV receptor CD46 (membrane cofactor protein) belongs to the family of membrane-associated complement regulatory proteins that serve as an important mechanism of self-protection against complement-mediated lysis. Tumor cells frequently overexpress CD46. These mechanisms contribute to the tumor selectivity of MV-Edm [30–32]. A third MV receptor, nectin 4, has recently been identified [33–35]. The expression of nectin 4 is localized to polarized epithelial cells. This localization supports the notion of cell tropism because MV also grows well in the epithelial cells of the respiratory tract. Nectin 4 may be used as a novel means of viral entry for both the wild-type and the vaccine strain of measles virus to enable spreading from cell to cell. Therefore, the MV-Edm may effectively spread between cells by either the CD46 or the nectin 4-dependent entrance [33–35]. In addition, numerous histopathological studies have indicated that MV has been detected in the neuronal cells, suggesting the existence of MV propagation in these cells [36]. In particular, MV has shown strong neuronal tropism and may cause acute and persistent encephalitis through an unknown pathway [37].

In contrast to the wild-type virus, which can cause a potentially serious disease, the vaccine and the Edmonston strains of the measles virus have an excellent safety record, with millions of administered vaccine doses that have significantly decreased the incidence, morbidity and mortality of measles worldwide [14]. Another advantage of the use of the MV-Edm as a vector is that the virus may be effectively engineered to express soluble marker peptides, such as CEA and beta-HCG, which may be employed as real-time correlates of viral gene expression in vivo. Furthermore, this virus expresses membrane proteins such as the sodium iodine symporter, which allows for radionuclide imaging with assessment of viral localization and spread over time [38,39]. The serum CEA

Table 1
Primers for real-time quantitative PCR.

Target	Length	Forward (5′-3′)	Reverse (5′-3′)
CD46	132 bp	TGT TTG AAT GCG ATA AGG	TTG AGA CTG GAG GCT TGT
Nectin 4	108 bp	ATC GCC GCA CTC TTG T	GGT CAG CTC CTC CTC ATA T

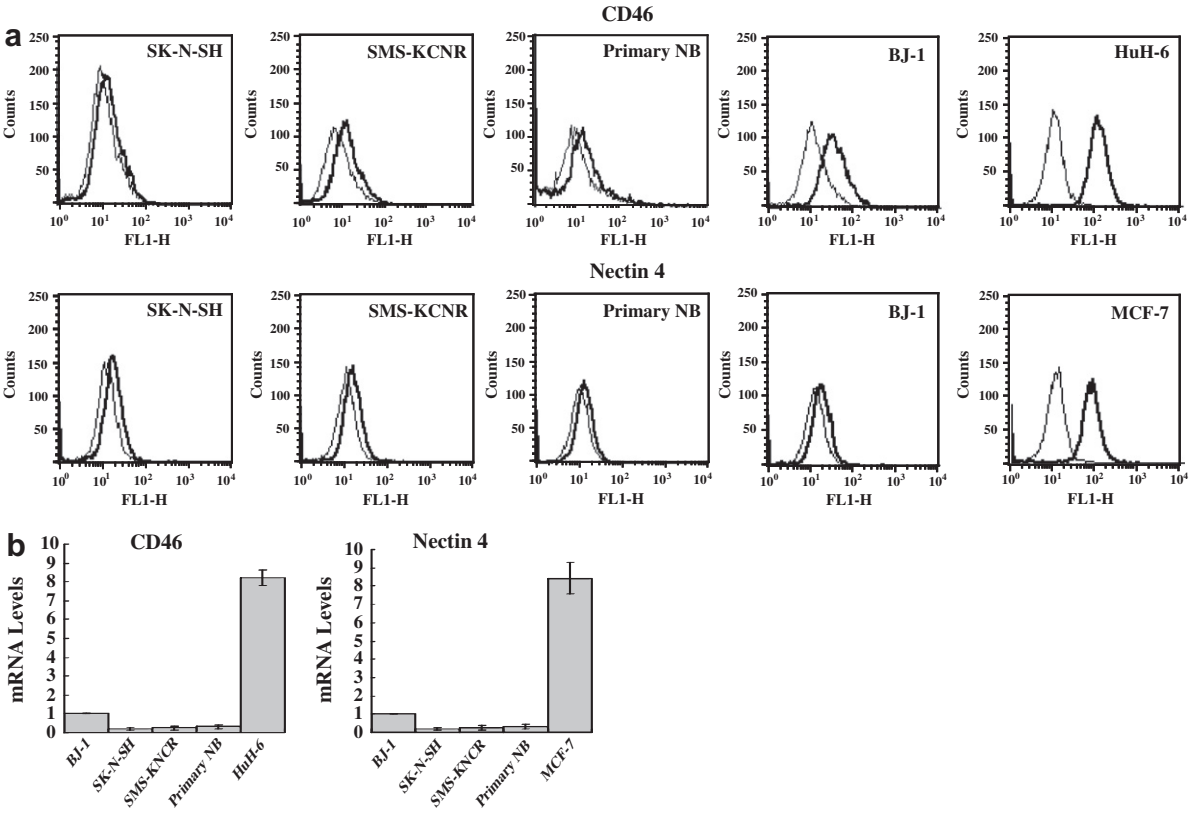

Fig. 1. Expression of CD46 and nectin 4 receptors in human NB cells and normal skin fibroblast cell lines. (a) Relatively lower levels of CD46 and nectin 4 receptors were observed in the human NB cell lines SK-N-SH and SMS-KCNR and in the primary NB cells, as well as in the normal skin fibroblast cell line BJ-1. The analysis was performed by flow cytometry. The thin histograms show the measured fluorescence of cells incubated with an isotype control (detailed), and the thick histograms represent cells labeled with an anti-CD46 or nectin 4 fluorescein isothiocyanate antibody. HUH-6 and MCF7 cells were used as positive controls. (b) The total RNA from the NB cells was isolated. The CD46 and nectin 4 mRNA levels were measured using real-time quantitative PCR. Each value is normalized to that in BJ-1, which was set to a ratio of 1, and represents the mean ± SD. The mRNA expression levels of CD46 and nectin 4 in the NB cells were much lower than those in the BJ-1 cells, and the results strongly supported the flow cytometry findings.