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Original Articles

Combination of vaccine-strain measles and mumps virus synergistically kills a wide range of human hematological cancer cells: Special focus on acute myeloid leukemia

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ABSTRACT

Through combining vaccine-derived measles and mumps viruses (MM), we efficiently targeted a wide range of hematopoietic cancer cell lines. MM synergistically killed many cell lines including acute myeloid leukemia (AML) cell lines. Further investigation suggested that enhanced oncolytic effect of MM was due to increased apoptosis induction. In an U937 xenograft AML mouse model, MM displayed greater tumor suppression and prolonged survival. Furthermore, MM efficiently killed blasts from 16 out of 20 AML patients and elicited more efficient killing effect on 11 patients when co-administered with Ara-C. Our results demonstrate that MM is a promising therapeutic candidate for hematological malignancies.

Introduction

Oncolytic virotherapy is a promising prospect to complement current therapeutic strategies in oncology, since cancers resistant to conventional treatment regimens are unlikely to be cross-resistant to oncolytic viruses (OLVs). Currently, numerous OLVs, including adenovirus [1–3], reovirus [4], measles virus [5], Newcastle disease virus [6], Seneca Valley virus [7], retrovirus [8], vaccinia virus [9], herpes simplex virus [10] and Coxsackie virus A21 [10] are in or have completed clinical trials for a variety of solid malignancies with encouraging results.

Given the heterogeneity associated with hematological malignancies, combination therapy is a rational option widely practiced in oncology. Apart from two reports on solid tumors [11,12], the combinatorial use of OLVs for hematological malignancies has not been evaluated to date. OLVs generally exhibit different modes of infection [13]. Consequently, a malignant cell is likely to be susceptible to infection by different viruses concurrently. Thus, it is anticipated that through combining different viruses, efficiency of OLV therapy might be improved.

Live-attenuated vaccine-strain viruses with well-established clinical and safety profiles can be combined to circumvent the potentially greater risk associated with combination therapy. An ideal candidate is the measles virus (MeV)-mumps virus (MuV)-rubella virus (RV) (MMR) vaccine, which has an excellent safety record. Notably, the virological and clinical features of MeV and MuV have been relatively well characterized. Vaccine strain MeV is known to preferentially infect various malignant cell types due to overexpression of CD46 [14]. Additionally, MeV has demonstrated activity against lymphomas [15], multiple myeloma [16], ovarian cancer [17], glioblastoma multiforme [18], breast cancer [19], hepatocellular cancer [20], head and neck squamous cancer [21], prostate cancer [22] and pancreatic cancer [23]. Clinical evidence

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also indicates that wild-type MuV can suppress the growth of various cancers without causing serious complications [24].

Here we propose a novel strategy to potentially expedite preclinical and clinical evaluations: the combined use of different vaccine-strain viruses with established safety records. We chose to evaluate a combination of MMR vaccine-derived MeV and MuV (MM), and focused on evaluating their target range, oncolytic effect, and the mechanism of cancer cell killing. We also investigated the oncolytic effect of MM *in vivo* using an U937 AML xenograft tumor model and *ex vivo* using primary blast cells from AML patients.

Materials and methods

Cell lines

Vero cells and human AML cell line THP-1 were kindly provided by Dr. Justin Chu Jiang Hann (Department of Microbiology, National University of Singapore [NUS], Singapore) and Dr. Subhash Vasudevan (Duke-NUS Graduate Medical School, Singapore) respectively. Human T cell leukemia (TCL) cell lines (Jurkat, MT-1, MT-2 and MT-4), AML cell line (U937), T lymphoma cell line (HuT 102) and B lymphoma cell lines (Raji and Namalwa) were maintained in our laboratory. Human promyelocytic leukemia cell line HL-60 was kindly provided by Prof. Huang De Jian (Department of Chemistry, NUS, Singapore). All media were supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Life Technologies, CA). Vero cells were cultured in M199 media (Biowest, France). All other cell lines were maintained in RPMI-1640 media (Invitrogen Singapore Pte Ltd, Singapore).

Infection and cell viability assay

To quantify oncolytic effect, 5000 cells in 100 μl RPMI-1640 were plated in 96-well clear-bottom white-walled plates and then infected with MeV, MuV or MM, which are isolated from MMR vaccine as shown in the Supplementary information, at indicated multiplicity of infection (MOI) in a final volume of 200 μl. Each virus infection was performed in triplicate. Cell viability was detected on day 3 and 6 post infection (P.I.) using the CellTiter-Glo® Luminescent cell viability assay (Promega, WI) according to the manufacturer's instruction. Results were expressed as percentage of viable cells relative to non-infected controls.

To determine the effect of combination (i.e. MM), 12,500 Jurkat and U937 cells in 500 μl RPMI-1640 were infected with MeV and MuV at a series of MOIs (1, 0.25 and 0.06) in 48-well plates. Cell viability was quantified on day 3 (Jurkat) or day 6 (U937) as described above. Synergism was identified using the median-effect principle of Chou and Talalay [25]; by calculating the combination-index (CI) values using CalcuSyn software (Biosoft, United Kingdom). CI indicates the degree of interaction between two or more agents; whereby values of 1 denote an additive interaction, >1 antagonism and <1 synergy.

Flow cytometric analysis

Annexin-V/PI staining was used for apoptosis detection. Cells were harvested and washed once in Annexin-V binding buffer (Biolegend, CA). Cells were then resuspended in 100 μl Annexin-V binding buffer containing 1 μl of Annexin-V-Alexa Fluor 647 (Biolegend, CA) and incubated at room temperature for 15 min. Finally, 300 μl Annexin-V binding buffer containing 1 μl of PI (0.5 mg/ml) (Biolegend, CA) was added. All samples were assayed using the BD LSR-Fortessa, and data were analyzed using FLowJo 9.3.2 (Treestar Inc. OR).

Subcutaneous AML xenograft model

Six-week-old male BALB/c nude mice (Sigma, USA) were kept under pathogen-free conditions in accordance with Animal Center Guidelines. The procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of NUS. Five million U937 cells in 50 μl Matrigel (BD Biosciences, CA) were subcutaneously implanted in the right flanks of anesthetized mice. When tumors reached a diameter of 5–10 mm, the mice were divided into four groups (5 mice per group). Multiple intra-tumoral injections of MeV, MuV or MM (1.0×10^5 $TCID_{50}$) were administered twice a week for 3 weeks. Control injections were with equivalent amounts M199 media. Tumor volumes were measured twice a week with slide calipers and calculated as follows: tumor volume (mm^3) = length × $width^2$ × ½.

Isolation and treatment of human PBMCs

Ethics clearance for this study was obtained from the Domain Specific Review Board (Domain B), National Healthcare Group, Singapore. Informed consent was obtained from patients as a part of a comprehensive leukemia repository at the National University Hospital (NUH, Singapore). PBMCs were isolated from heparinized peripheral blood obtained from healthy adult donors or AML patients by centrifugation on lymphocyte separation medium (Sigma-Aldrich, MO). Cells at the interface were collected and washed twice in cold RPMI-1640. One hundred thousand PBMCs were infected with MM at a MOI of 1, with or without 1 μM Ara-C (Sigma-Aldrich, MO), in 96-well clear-bottom white-walled plates. Cell viability was determined on day 6 P.I. as described above.

Statistical analysis

Results were analyzed using Prism software (GraphPad Software, CA). Student's t test was used to compare the differences between MM-treated and MeV- or MuV-treated groups. The following symbols were used to denote statistical significance: $^*P \leqq 0.05$; $^{**}P \leqq 0.01$; and $^{***}P \leqq 0.001$.

Results

MMR infection produced cytopathic effects (CPE) in a wide range of hematological malignancy cell lines

We initially evaluated the oncolytic effect of MMR for combination OLV therapy by infecting cell lines of various hematological malignancies (3 AML, 4 TCL, 1 T lymphoma and 2 B lymphoma cell lines). We observed CPE in all cell lines tested (Fig. 1). However, RV was undetectable by RT-PCR in all cell lines tested except HL-60 and Namalwa (Supplementary Fig. S1), suggesting that RV does not infect or replicate in most tested cell lines. Therefore, we evaluated the effect of MM combination in subsequent experiments.

MM also targeted a wider range of cancer cell lines

In deciding the MOI used in subsequent experiments, we first quantified the oncolytic effect of MeV, MuV and MM at different MOIs using U937 cells. As expected, greater cell killing was observed when MOI increased (Supplementary Fig. S2).

We evaluated if MM targeted a wider range of hematological malignancies compared to MeV or MuV using a panel of 10 cancer cell lines. To ensure the killing effect of both viruses on tested cell lines, we administered a high concentration for each virus at a MOI of 1. MeV showed strong oncolytic effect against TCL, T and B lymphoma cell lines; but a weak effect on the AML cell lines (Fig. 2 and Supplementary Fig. S3). MuV was most effective against T lymphoma cell lines followed by TCL, AML and B lymphoma cell lines (Fig. 2 and Supplementary Fig. S3). However, MM was invariably more effective in killing all cell lines used (Fig. 2 and Supplementary Fig. S3). These results show that MM efficiently targets a wider range of hematological malignancies compared to MeV or MuV.

MM showed greater oncolytic effect on several cell lines compared with single virus, and displayed oncolytic activity against U937 and Jurkat cells in a synergistic manner

MM combination could target various types of cancer cells. Further analysis showed enhanced killing compared to individual viruses not only in U937 and Jurkat but also THP-1, HL-60, MT-1, MT-2, MT-4 and HuT-102 cell lines at day 6 as shown in Fig. 3, suggesting that MM may synergistically kill target cells.

To investigate whether MM combination has synergistic effect, we evaluated various combinations of concentrations using the Chou–Talalay method, which was previously used to assess synergism of oncolytic viruses [12]. Synergistic oncolytic effect was observed for almost all MM combination doses (Table 1). Notably, MOI 1 for each virus as combination dose showed the most potent and higher synergistic oncolytic effect in U937 and Jurkat cells: as reflected by CI values of 0.056 and 0.610 respectively. Therefore, this MOI was used for subsequent MM studies unless otherwise stated.