Using the Cow Pea Mosaic Virus (CPMV) As a Bio-Imaging Tool

Cancer diagnostic and therapeutic methods, such as radiation and chemotherapy, eliminate cancer cells as well as beneficial cells and may cause side effects that are uncomfortable for the patient. The focus of this project was to develop a novel diagnostic vehicle to target tumor cells using the cowpea mosaic virus (CPMV) as a scaffold. We attached indicators for magnetic resonance imaging (MRI) and fluorescence imaging to the virus, which allowed us to perform non-invasive detection of tumor cells within a patient's body. Because of the successful modification of CPMV with near infrared fluorescent dye (NIRF dye) and a terbium (Tb) metal complex, it may be possible to use CPMV as a new diagnostic or therapeutic tool for cancer.

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Introduction

According to the cancer.org website, approximately one out of every two American men and one out of every three American women will have some type of cancer at some point during their lifetime. Cancer patients are given numerous treatments like chemotherapy and radiation to destroy the tumor cells, but such treatments are also fighting off beneficial cells that control weight, hair growth, and numerous other properties that help maintain a person's health. Our research was to develop a new treatment that will selectively deliver NIRF dye and metal complexes to tumor cells so they may be seen more clearly using fluorescence imaging or MRI for the metal complex.

Due to the capability of the cow pea mosaic virus (CPMV) to remain stable in pH 4.0 to 10.0, its availability, and its ability to chemically modify cells,[1] studies have been conducted using it as an aid in early diagnosis. CPMV may be attached to probes that will be transported to tumor cells selectively as a diagnostic agent. When diagnostic, therapeutic, and targeting reagents are delivered to certain tumor cells, they may specifically target, image, and treat the tumor cells while allowing healthy cells to remain unharmed during cancer treatments.

Methods

In order to attach the indicators to the virus several steps had to be taken. The viruses were purified and observed through the ultra-violet visible spectrophotometer (UV-Vis) for observation. A dye or terbium was added and the virus sample was purified and analyzed once more. The virus purification was completed to gather the virus particles off the leaves and destroy the excess plant material that was not needed. Several steps were performed: the blending of the frozen leaves in pH 7.0 K-phosphate buffer (0.1 M); the adding of mercaptoethanol to the solution; centrifuging the virus samples several times; and later resuspending the virus in buffer.[2] Following our previous study,[3,4] the virus was then incubated with NIRF dye and Tb complex[5] at 4°C over-night. The non-reactive chemical reagents were then removed with gel filtration chromatography.

Data Analysis and Results

Thirty terbium metals were attached to each virus (Figure 1). UV-Vis was used to detect the concentrations of viruses and terbium complexes. We further confirmed that the terbium modified CPMV remained stable as revealed with transmission electron microscopy (TEM), which established the basis of using CPMV to deliver MRI reagents (eg, Gd compounds) for in vivo imaging.

Similarly, 28 NIRF dyes were attached per virus and the viral products remained intact. These products have been sent to our collaborators for fur-

$$\frac{A_{Tb\ or\ dye}}{\varepsilon_{Tb\ or\ dye}} \times \frac{MW\ (100{,}000)\ protein}{Virus\ concentration} = \frac{terbium\ or\ dye}{subunit} \times \frac{60\ subunits}{particle} = \frac{terbium}{particle}$$

Fig 1. Equation used to attach 30 terbium metals to each virus

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ther fluorescence imaging study of tumor cells.

CONCLUSIONS

The Cowpea Mosaic Virus was used as a scaffold for indicators aiding in early detection of cancer. By having dyes or metal complexes attached to it, CPMV was employed as a vector of indicators for enhanced MRI and fluorescence imaging. In our study, CPMV was purified and chemically modified near infrared fluorescent dye and terbium moieties, which will be used in non-invasive tumor detection with fluorescent imaging or MRI. We will further engineer targeting groups on the surface of viral particle to achieve selective delivery of therapeutic and diagnostic agents to tumors.

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