Plant viruses become useful material for cancer theranostics

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Amid various diseases, cancer remains one of the leading causes of mortality worldwide. In India, it is among the top three most common diseases, with ~10 million cases reported in 2019. Cancer causes a million deaths per year, including those below 10 years of age. Despite advances, diagnostic procedures and treatments, the overall survival rate from cancer has not significantly improved in the past two decades. The development of imaging and therapeutic agents that target cellular and molecular activities of the disease is highly desired, since they will allow detection and treatment at early stages.

Recently, supramolecular systems (micelles, liposomes, polymers, etc.) have been explored as delivery vehicles for imaging and as therapeutic agents for cancer. Among the supramolecular designs studied, viruses have gained significant attention with several advantages over artificial materials[1]. Viruses are non-living, parasitic–pathogenic agents causing severe diseases in all living forms on earth. They have been used for many purposes, from vaccine to gene therapy[2]. Recently, researchers have found that viruses are useful nanoscale materials for various applications like electronics, sensors, biocatalysts and targeted therapeutic delivery[1].

Plant virus-based systems, in particular, are among the most advanced and exploited for their potential use as bioinspired structured nanomaterials and nano-vectors. Plant viruses are particularly suitable for nanoscale applications and can offer several advantages. They are structurally uniform, robust, biodegradable and easy to produce. There are multiple examples of how plant–virus–nanoparticles have been functionalized to attach molecules to their external surfaces and use their internal cavities to load drugs and other molecules as cargo molecules[3]. This plasticity in terms of nanoparticles engineering is the ground on which multivalency, payload containment and targeted delivery can be fully exploited[4]. According to Lomonossoff[5], the capsids of most plant viruses are simple and robust structures consisting of multiple copies of one or a few types of protein subunits arranged with either icosahedral or helical symmetry. The capsids are produced in large quantities either by the natural infection of plants or by the expression of the subunit(s) in various heterologous systems. Given their relative simplicity and ease of production, plant virus particles or virus-like particles (VLPs) have attracted interest over the past 20 years for applications in both bio- and nanotechnology[5]. As a result, plant virus particles have been subjected to both genetic and chemical modification, used to encapsulate foreign material and have themselves been incorporated into supramolecular structures. Significantly, plant viruses studied are not human pathogens, which have no natural tendency to interact with human cell-surface receptors[6]. However, focus is on the virus protective structure made up of multiple coat-protein monomers called a capsid. Plant viruses are mostly made of a protein envelope and genetic material. The viral capsid has attracted interest in biomedical applications because of its nanoscale size, symmetrical structural organization, load capacity, controllable self-assembly and ease of modification. Viruses are essentially naturally occurring biomaterials capable of self-assembly with a high degree of precision.

Virus nanoparticles (VNPs) have been used as 'nanocargos' for target delivery at cancer sites. However, the interior of the virus capsid is used to encapsulate with different imaging molecules and antitumour drugs by the gating mechanism[7,8]. The gating mechanism mimics the natural in the cytosol, where viral particles get assembled and disassembled in favour of cytosolic pH and ionic strength. This event can occur by simple dialysis against different pH buffers[6]. The exterior of the virus capsid has been modified and functionalized using bio-conjugation chemistry to target specific cancer sites. Since it is a protein, there is a large availability of featured amino acid sites for bioconjugation chemistry. Using bioinformatics tools (Molsoft-ICM Pro), the coat protein available conjugation sites also can be predicted and skillfully managed[7]. Extensive research works on virus nanoparticles are now available to discuss the efficient modification of whole virus particles for cancer target delivery. Virus particles of plant origin are considered non-infectious and non-hazardous to humans and other mammals. Because nowhere reported the toxicological impact on humans who consume foods infected by plant viruses. We can obtain vast quantities of plant virus particles from infected plants by sucrose gradient ultracentrifugation.

Another interesting aspect in the modification of viruses for target delivery is the VLP. A VLP is a hollow, a self-assembled, spherical protein structure typically assembled by multiple copies of protein monomers to a viral-like structure without nucleic acid. VLPs are often produced in large quantities by heterologous expression systems such as *Escherichia coli* and yeast. They are also a good material for functional tuning however, chemical and genetic modification can be used to generate numerous functionalities by adding drugs, toxins, imaging reagents, epitopes and specific targeting peptides to the internal and external surfaces. For example, the specific VLPs can be obtained by coat protein expression in *E. coli* or yeast expression systems. Further, they can be self-assembled like VLPs under *in vitro* conditions using an assembly buffer that contains pH and ionic strength mimicking the intracellular buffering system[7].

Similarly, *in vitro* self-assembly mechanisms can be exploited to selectively entrap materials within the VLPs (Figure 1). The interior cavity of VLPs is covalently entrapped with imaging moieties (iron oxide nanoparticles gadolinium ion, magnetic nanoparticles and quantum dots) and antitumour drugs (5-fluorouracil)[9]. The outer surface of VLPs can be PEGylated using NHS (*N*-hydroxysuccinimide) chemistries, while the remaining interface between adjacent coat proteins in capsomers can be bioconjugated with folate ligands targeting upregulated folate receptors (FR) in FR-positive ovarian cancer cells[10]. All these events were carefully studied by size exclusion chromatography and electron microscopy. These programmable 'VLP-nanocarriers' will allow cancer cell-specific smart delivery of both imaging and therapeutic drugs. Several plant-based viruses such as *Brome mosaic virus* (BMV), *Cowpea chlorotic mottle virus* (CCMV), *Cowpea mosaic virus* (CPMV), *Potato virus X* (PVX) and *Tobacco mosaic virus* (TMV) have been systematically studied and used as VLPs for many applications.

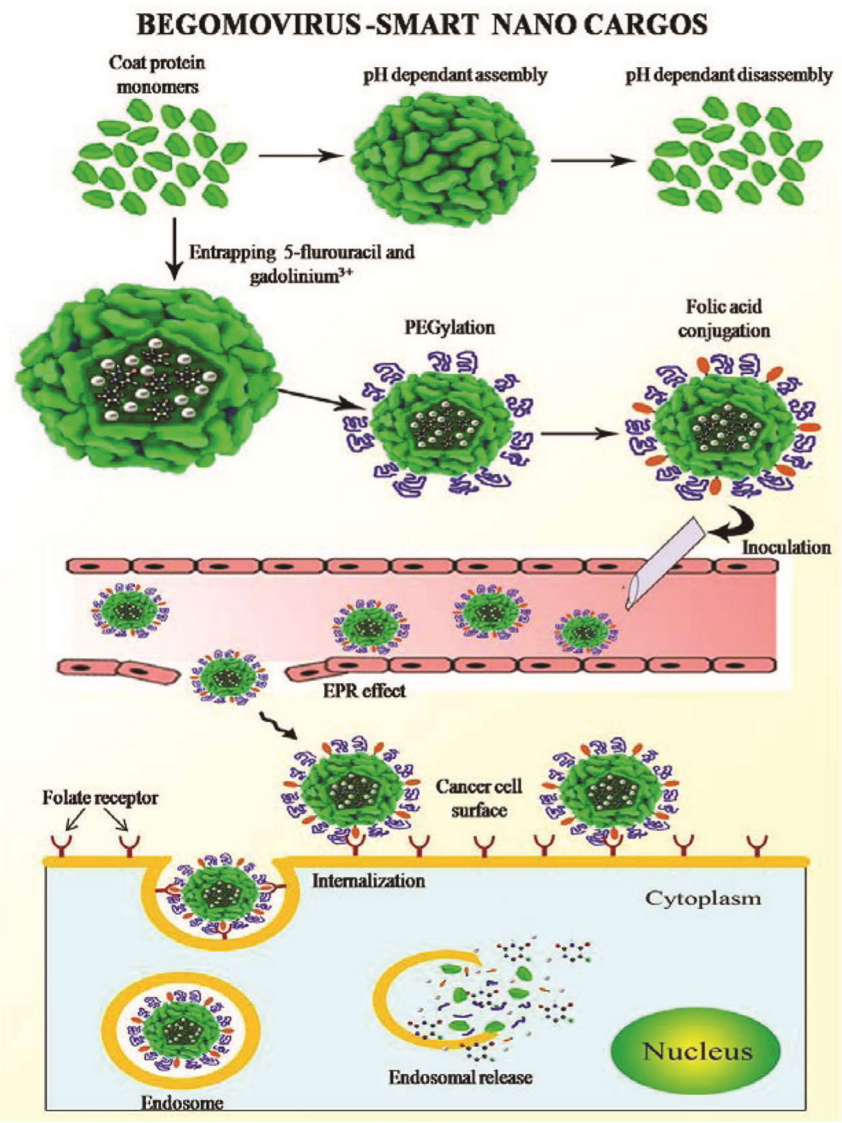

Figure 1. Graphical abstract.

We have studied one of the plant pathogenic viruses of Begomovirus isolates of *Squash leaf curl China virus* for cancer theranostics[7]. The virus nanocarrier-mediated theranostics offers concurrent delivery of therapeutic payloads and diagnostics, enabling real-time monitoring of drug distribution, pharmacokinetics and consequent pathological manifestation in response to the drug. This ideology could be a potential platform to establish targeted imaging and therapy for a wide range of cancers in the near future without making patients suffer the toxic side effects, because cancer chemotherapies maintain some success stories and some terrifying ones which result in long-term side effects in patients.

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ACKNOWLEDGEMENTS. I thank Prof. K. Kathiravan, Department of Biotechnology, University of Madras, Chennai and Dr R. Viswanathan, Head, Division of Crop Protection, ICAR-Sugarcane Breeding Institute, Coimbatore, for constant support during the study and Science and Engineering Research Board, Department of Science and Technology, Government of India for the award of National Postdoctoral Fellowship (PDF/2020/002415).

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