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The Cancer Control Challenge:
Better Understanding It to Better Face It

TRANSIENT EXPRESSION OF CPMV VIRUS-LIKE PARTICLES (VLPS) IN PLANTS: A THERAPEUTIC TARGETING STRATEGY FOR GASTRIC CANCER

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Introduction: Gastric cancer (GC) is the fifth leading cause of cancer-related death worldwide, with approximately one million new cases annually. In Northern Brazil, its incidence and mortality exceed the national average. GC is highly heterogeneous, and late diagnosis frequently leads to metastasis and resistance to conventional therapies, reinforcing the need for targeted therapeutic strategies. Virus-like particles (VLPs) represent a promising alternative, as they are virus-derived structures composed of structural proteins that mimic native viral architecture without containing viral genetic material. VLPs can be engineered to encapsulate therapeutic molecules and target tumor cells through surface bioconjugation of ligands. In this context, plant viruses such as cowpea mosaic virus (CPMV) are attractive candidates due to their inability to infect mammalian cells, ensuring biosafety. RNA-free CPMV particles therefore constitute a promising platform for active delivery of therapeutic agents. Among available production systems, transient expression in plants—particularly via *Agrobacterium tumefaciens* infiltration in *Nicotiana benthamiana*—stands out for its rapidity, cost-effectiveness, and low risk of contamination by animal pathogens. **Objectives:** To express and characterize virus-like particles derived from cowpea mosaic virus (CPMV) in *Nicotiana benthamiana* as a potential targeting strategy for gastric cancer therapy. **Methods:** Suspensions of *Agrobacterium tumefaciens* carrying the plasmid pEAQexpress-VP60-24K were infiltrated into *Nicotiana benthamiana* leaves. Capsid proteins were transiently expressed, extracted, clarified with n-butanol, and precipitated using polyethylene glycol (PEG). The particles were subsequently purified by ultracentrifugation. Quantification was performed by spectrophotometry (NanoDrop) using the Lambert–Beer law ($A = \varepsilon \cdot c \cdot l$). Structural characterization was carried out by polyacrylamide gel electrophoresis (SDS-PAGE), dot blot, and Western blot assays using anti-CPSMV antibodies. **Results:** CPMV VLPs were successfully expressed and recovered six days after infiltration. The determined concentration of the analyzed sample

was 2.546 mg/mL, which was subsequently used in downstream experiments. SDS-PAGE analysis (4–12% polyacrylamide gel) confirmed the presence of large (L) and small (S) capsid proteins with expected molecular weights of approximately 42 kDa and 24 kDa, respectively, for both CPMV VLPs and cowpea severe mosaic virus (CPSMV), used as a positive control. Dot blot assays showed qualitative immunoreactivity for both CPSMV and CPMV VLPs when probed with anti-CPSMV antibodies. Western blot analysis further confirmed the presence of the small capsid protein at 24 kDa. **Conclusion:** Transient expression in *Nicotiana benthamiana* proved to be an efficient and reliable platform for the production of recombinant viral proteins with potential oncological applications. The versatility of CPMV-derived VLPs, combined with their capacity for molecular modification and tumor targeting, positions this platform as a promising strategy for the development of innovative therapeutic approaches for gastric cancer.

Keywords: Gastric adenocarcinoma; transient expression; plant viruses; plant viral nanoparticle