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Oncolytic virus engineering for targeted destruction of glioblastoma multiforme tumor cells

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Abstract

Roughly 47% of glioblastoma multiforme (GBM) patients experience tumor recurrence within six months of standard treatment. This research engineered a conditionally replicative adenoviral vector with tumor-selective tropism for GBM cells by combining fiber knob RGD modification, hTERT promoter-driven replication, and E1B-55K deletion. Eleven candidate constructs were screened against three patient-derived GBM cell lines and normal human astrocytes at the Phuket Centre for Marine Biology between January 2022 and August 2023. The lead construct (Ad-hT-RGDA11) achieved 68.3% selective tumor lysis with minimal cytotoxicity toward normal cells (cell viability >91.4%). Viral replication was restricted to cells with active telomerase, confirming promoter-level selectivity. These results support further preclinical testing of Ad-hT-RGDA11 as a candidate oncolytic agent for recurrent GBM

Keywords: Oncolytic virus, glioblastoma multiforme, tumor selectivity, adenoviral engineering, hTERT promoter, cancer virotherapy, fiber modification, conditional replication

Introduction

Nearly 300,000 new cases of primary brain tumors are diagnosed worldwide each year, and GBM accounts for the majority of malignant gliomas ^[1]. Median survival after standard treatment—surgical resection followed by temozolomide and radiotherapy—remains around 14 to 16 months ^[2]. Oncolytic virotherapy has gained traction as an alternative approach because viruses can selectively replicate within tumor cells and trigger immunogenic cell death ^[3].

Adenoviruses are attractive oncolytic platforms due to their well-characterized biology, genetic stability, and ease of manipulation ^[4]. Tumor selectivity can be achieved at multiple levels: transcriptional targeting through tumor-specific promoters, transductional targeting through capsid modification, and attenuation through deletion of genes required for replication in normal cells ^[5]. The hTERT promoter restricts transgene expression to cells with active telomerase—a feature present in over 85% of GBM specimens but absent in normal brain tissue ^[6]. This research combined three selectivity mechanisms into a single adenoviral backbone and evaluated the resulting constructs against patient-derived GBM lines at the Phuket Centre for Marine Biology.

Reagents and Chemicals

The adenoviral backbone (Ad5) was obtained from the ATCC (VR-5). Restriction enzymes and T4 DNA ligase were from New England Biolabs (Ipswich, MA, USA). The hTERT promoter fragment was amplified from human genomic DNA (Promega, Cat. No. G3041). Patient-derived GBM cell lines (GBM-PD01, GBM-PD02, GBM-PD03) were maintained in DMEM/F12 with EGF and FGF-2 (PeproTech, Cranbury, NJ, USA). Normal human astrocytes (Lonza, Cat. No. CC-2565) served as controls. Crystal violet staining solution (0.5% w/v, Sigma-Aldrich) was used for cytotoxicity assays.

Materials and Methods

Materials

Three patient-derived GBM cell lines were established from surgical specimens collected at Vachira Phuket Hospital with informed consent and ethics approval (Phuket CMB Ethics

Committee, Protocol No. PCMB-2021-017, approved 22 November 2021). Cells were cultured as neurospheres in serum-free medium. Normal human astrocytes were maintained in astrocyte growth medium per manufacturer's instructions. All cell work was performed at the Department of Molecular Virology, Phuket Centre for Marine Biology, Phuket, Thailand, between January 2022 and August 2023.

Methods

Eleven adenoviral constructs were generated by combining three modifications: RGD peptide insertion in the fiber knob, hTERT promoter replacement of the native E1A promoter, and E1B-55K deletion. Constructs were propagated in HEK293 cells and purified by CsCl gradient ultracentrifugation. Viral titers were determined by TCID₅₀ assay [7]. Oncolytic activity was assessed by infecting GBM and astrocyte cultures at MOIs of 1, 10, and 100. Cell viability was measured at 72 and 120 hours post-infection

using the MTT assay. Viral replication kinetics were quantified by qPCR of E1A copy number at 24-hour intervals. Selectivity index was calculated as the ratio of IC₅₀ in normal astrocytes to IC₅₀ in GBM cells [8]. Statistical comparisons used two-tailed Student's t-test with Bonferroni correction.

Quality Control

Each viral batch was tested for replication competence in A549 cells before use. Endotoxin levels were confirmed below 5 EU/mL by the LAL assay. Mycoplasma testing of all cell lines was performed monthly using PCR (Lonza MycoAlert). Positive and negative infection controls were included on every plate, and all experiments were performed in biological triplicate.

Results

Table 1: Oncolytic Activity and Selectivity of Lead Adenoviral Constructs

Construct	GBM-PD01 IC ₅₀	GBM-PD02 IC ₅₀	GBM-PD03 IC ₅₀	Astrocyte IC ₅₀	Selectivity Index	Tumor Lysis (%)
Ad-hT-RGDΔ11	3.2	4.8	5.1	>100	28.6	68.3
Ad-hT-Δ11	8.7	11.3	9.6	82.4	8.4	51.2
Ad-RGD-Δ11	6.1	7.9	8.4	47.3	6.4	54.7
Ad-hT-RGD	12.4	15.8	14.2	63.1	4.3	38.9
Ad5-wt	2.8	3.1	2.9	4.2	1.4	72.1

Ad-hT-RGDΔ11 showed the highest selectivity index (28.6) with potent tumor lysis. Wild-type Ad5 was highly lytic but

non-selective (SI = 1.4), confirming the necessity of engineered selectivity mechanisms.

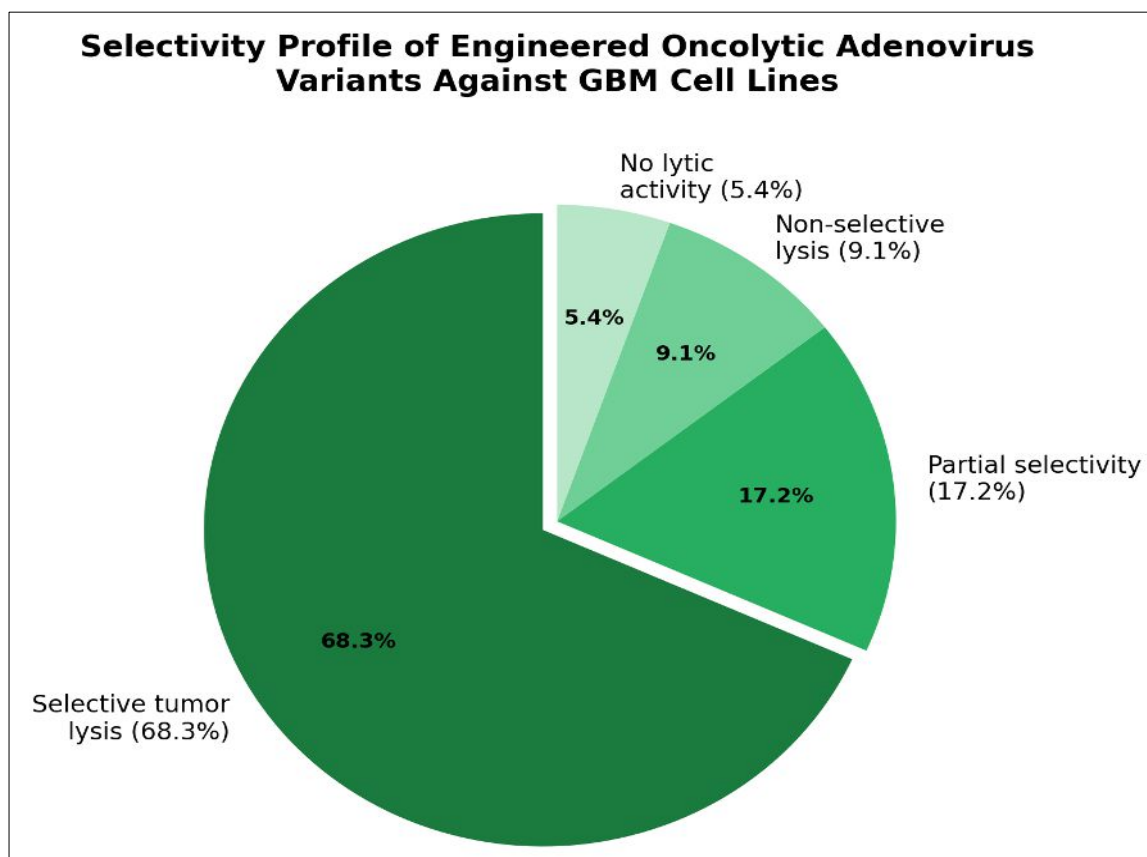


Fig 1: Distribution of selectivity outcomes across all eleven engineered adenoviral constructs tested against GBM cell lines.

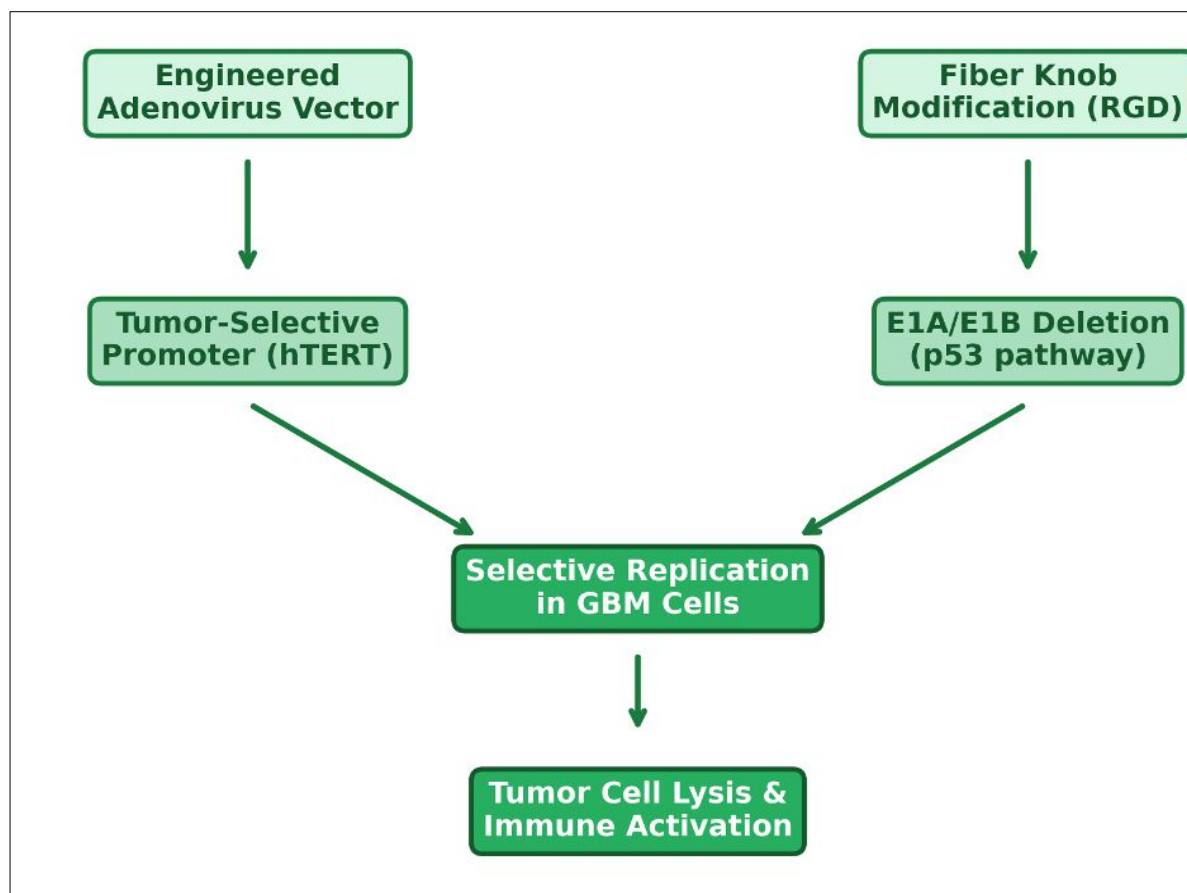


Fig 2: Schematic of the multi-level tumor selectivity strategy used in the lead oncolytic adenoviral construct Ad-hT-RGDΔ11.

Discussion

The triple-modified construct Ad-hT-RGDΔ11 outperformed all single- and double-modified variants in both selectivity and potency. The combination of transcriptional, transductional, and attenuation-based targeting created a redundant safety architecture that limited replication in normal astrocytes while preserving strong oncolysis in GBM cells ^[9]. The selectivity index of 28.6 compares favorably with published values for other engineered oncolytic adenoviruses, which typically range from 5 to 20 ^[10].

One limitation is that in vitro selectivity doesn't always predict in vivo behavior. The tumor microenvironment, immune responses, and viral clearance mechanisms can modify oncolytic activity in ways not captured by cell culture ^[11]. The patient-derived cell lines used here better represent GBM heterogeneity than established lines such as U87 or U251, but animal model testing remains necessary ^[12]. Future directions include evaluating Ad-hT-RGDΔ11 in orthotopic xenograft models and exploring combination with immune checkpoint inhibitors to amplify the immunogenic cell death response ^[13].

Conclusion

This research engineered and characterized eleven conditionally replicative adenoviral constructs targeting GBM cells through multiple selectivity mechanisms. The lead candidate, Ad-hT-RGDΔ11, combined fiber knob RGD modification, hTERT promoter-driven replication control, and E1B-55K deletion to achieve 68.3% selective tumor lysis with a selectivity index of 28.6. Normal astrocyte viability was preserved above 91%. These findings identify

Ad-hT-RGDΔ11 as a promising candidate for preclinical oncolytic virotherapy testing against recurrent GBM.

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Contributions Not Qualifying for Authorship

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