

HSV VECTOR EXPRESSING A VARIANT OF HAS2 GENE EXHIBITS ENHANCED ONCOLYTIC EFFECT AND TUMOR SUPPRESSOR RESPONSE

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Introduction

- The extracellular matrix (ECM) is a network of macromolecules surrounding cells, providing support and regulating cell growth and differentiation. Disruption of the ECM is common in cancer.
- Hyaluronan (HA) is an extracellular matrix (ECM) glycosaminoglycan which can either promote or suppress cancer cell growth, depending on its molecular weight and the cellular context. The naked mole rat (NMR) has a unique mutation in the *hyaluronan synthase 2* (*has2*) gene, increasing the production of the anti-tumorigenic high molecular weight HA (HMW-HA). The mutated *has2* gene is the key element in the exceptional cancer resistance of the species and it's also been shown to lower cancer incidence and increase the median and maximum lifespan in transgenic mice.
- We have developed an attenuated, replication-competent Herpes simplex virus (HSV) vector in which the cDNA of the *has2* gene variant XM_004838010.2 was cloned into $\gamma_134.5$ -locus of the HSV-backbone. Deletion of HSV gene $\gamma_134.5$ prevents lytic infection in healthy neurons, but not in cancer cells, presenting an ideal oncolytic gene therapy vector. The vector was designated as H1551.
- A similar HSV-vector (H0951) expressing *green fluorescent protein* (*gfp*) as the transgene was used as a control in the study. Both vectors have deletions in the $\gamma_134.5$ - and *UL44/gC* genes and a *luciferase* expression cassette insertion.
- We have previously shown that the H1551 vector shows an effective transgene expression and enhanced oncolytic effect on glioma cells [1].

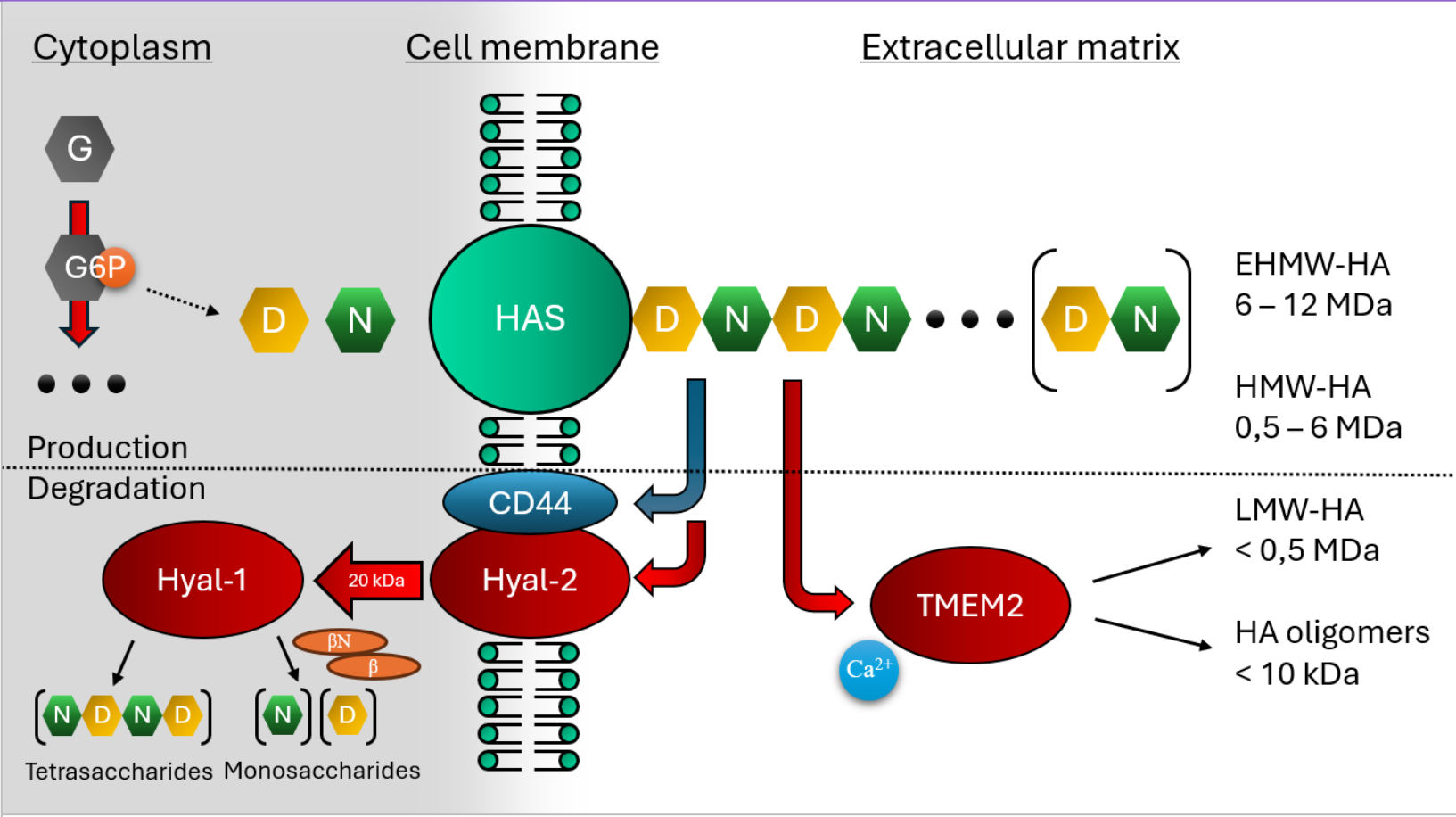


Figure 1: High molecular weight hyaluronan (HMW-HA) is produced on the cell membrane by hyaluronan synthase (HAS) enzymes. It is degraded in the extracellular matrix, on the cell membrane and in the cytoplasm. Abbreviations: G, glucose; G6P, Glucose-6-phosphate; D, D-glucuronic acid; N, N-acetylglucosamine; Hyal, Hyaluronidase; Ca²⁺, calcium ion; β , β -glucuronidase; β N, β -N-acetyl glucosamidase; TMEM2, Transmembrane protein 2; EHMW-HA, Extreme high molecular weight hyaluronan; LMW-HA, Low molecular weight hyaluronan. [2]

Methods

- U373MG glioma cells were infected with the H1551 vector (*has2*) and a control vector H0951 (*gfp*). The infection medium was collected to analyze the molecular weight distribution of HA produced by the infected cells and the cells were collected to analyze the tumor suppressor response at 24 hours post infection (hpi) and 48 hpi
- A pilot study *in ovo* was performed to analyze the effect of the vector on tumor growth. Tumors were inoculation into fertilized eggs at the embryonal day (ed) 7, infected at the ed 11 and the eggs were euthanized at the ed 14 (72 hpi).

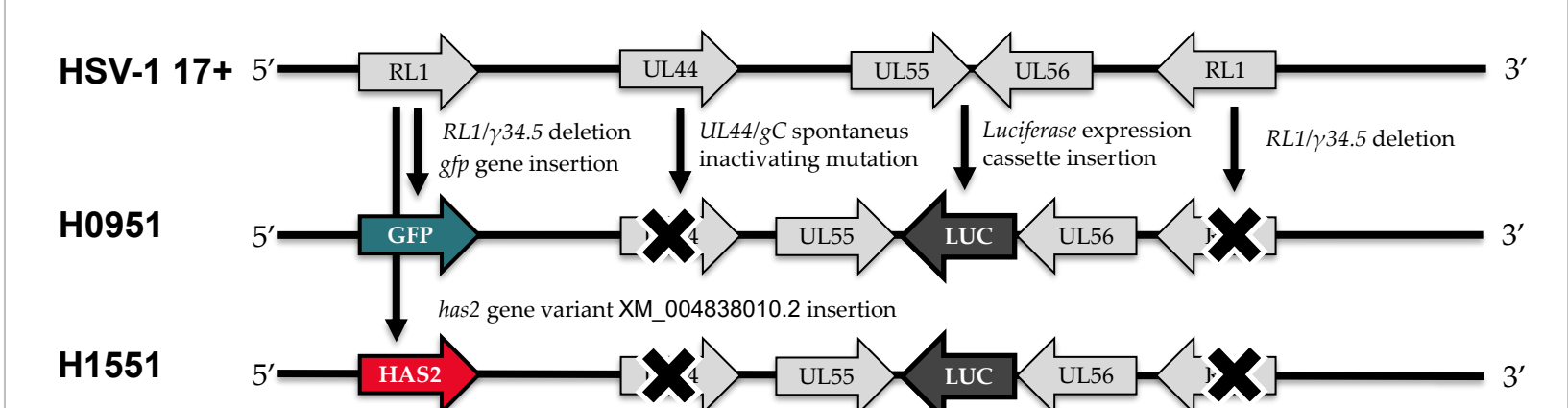


Figure 2: The HSV vectors H0951 and H1551 are $\gamma_134.5$ - and *gC*-negative transgenic vectors derived from the HSV-1 strain17+. [1]

Oncolytic effect [1]

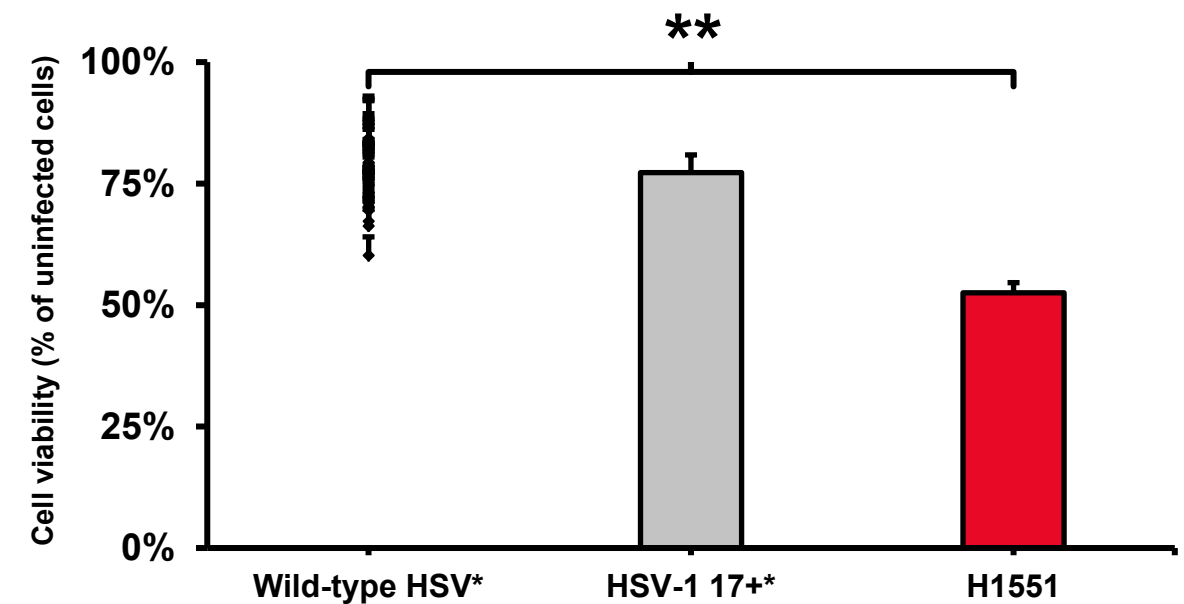


Figure 3: Cytotoxicity in glioma cells 96 hpi, measured by a luminescent assay. The viability was determined by comparing the signal derived from the infected cells to that derived from the non-infected cells. (n = 4) (* = [3], ** = statistical significance)

HA production

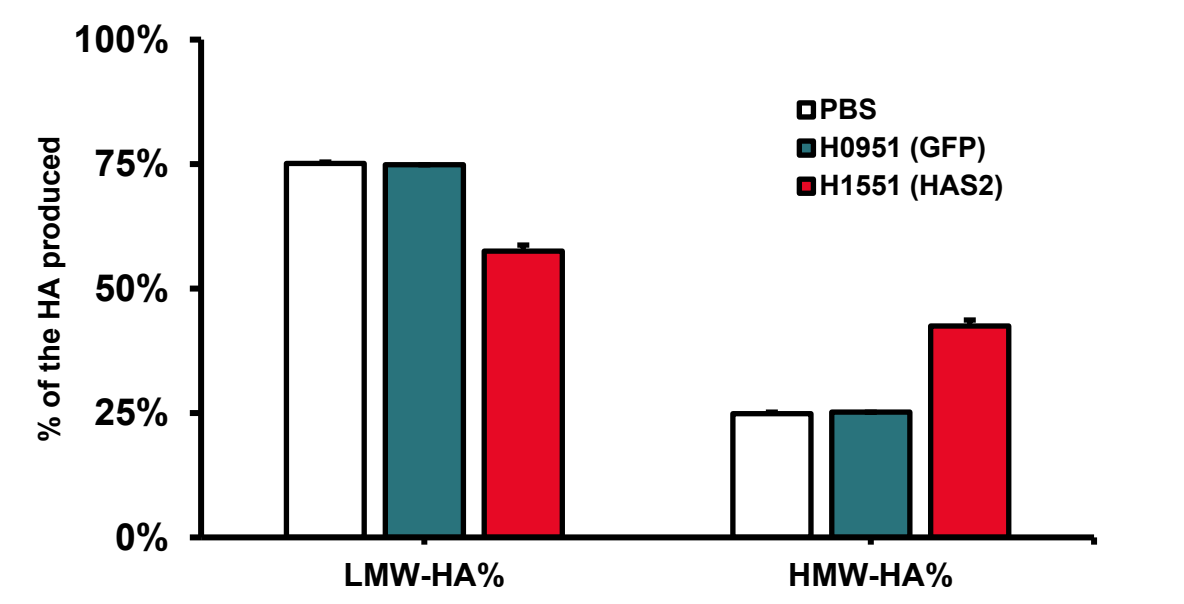


Figure 4: Molecular weight of the HA produced by U373MG cells infected with H1551 (*has2*) and control virus H0951 (*gfp*) at 48 hpi (n = 2). LMW-HA, Low molecular weight hyaluronan; HMW-HA, High molecular weight hyaluronan.

p53 expression

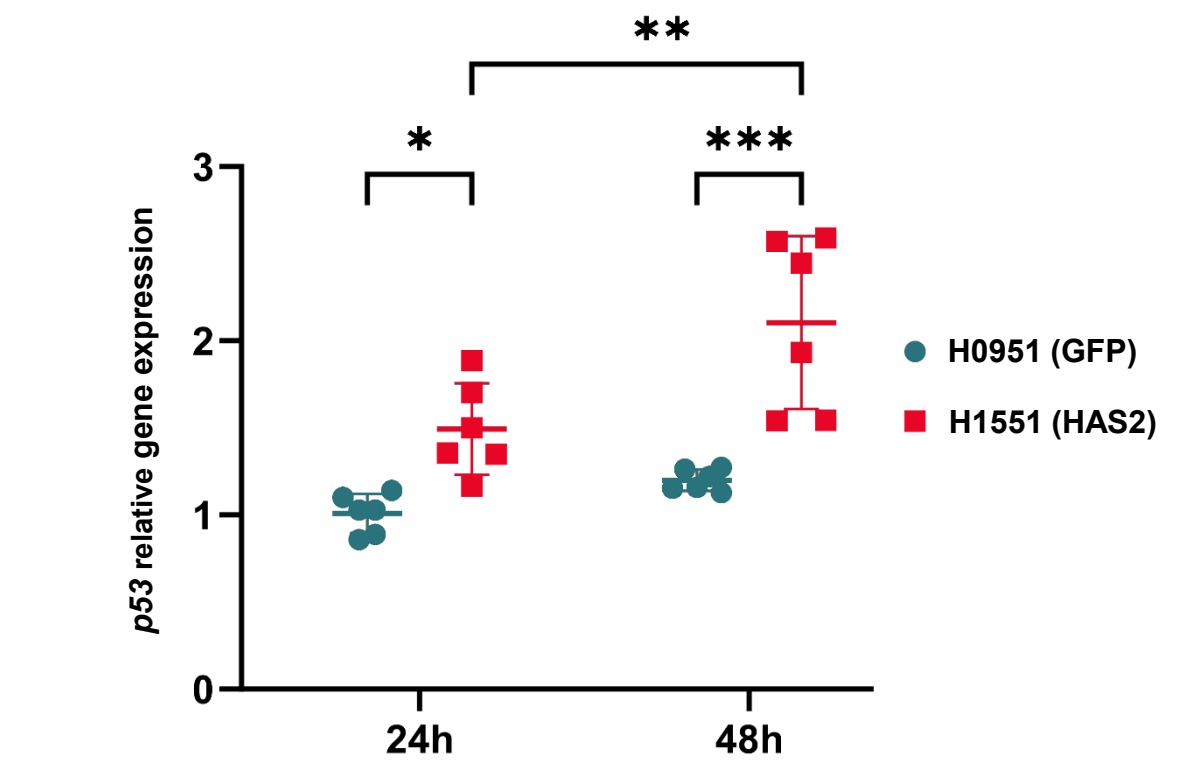


Figure 5: Expression of tumor suppressor gene *p53* in U373MG cells infected with H1551 (*has2*) and control virus H0951 (*gfp*). (*/**/**** = statistical significance)

Tumor growth *in ovo*

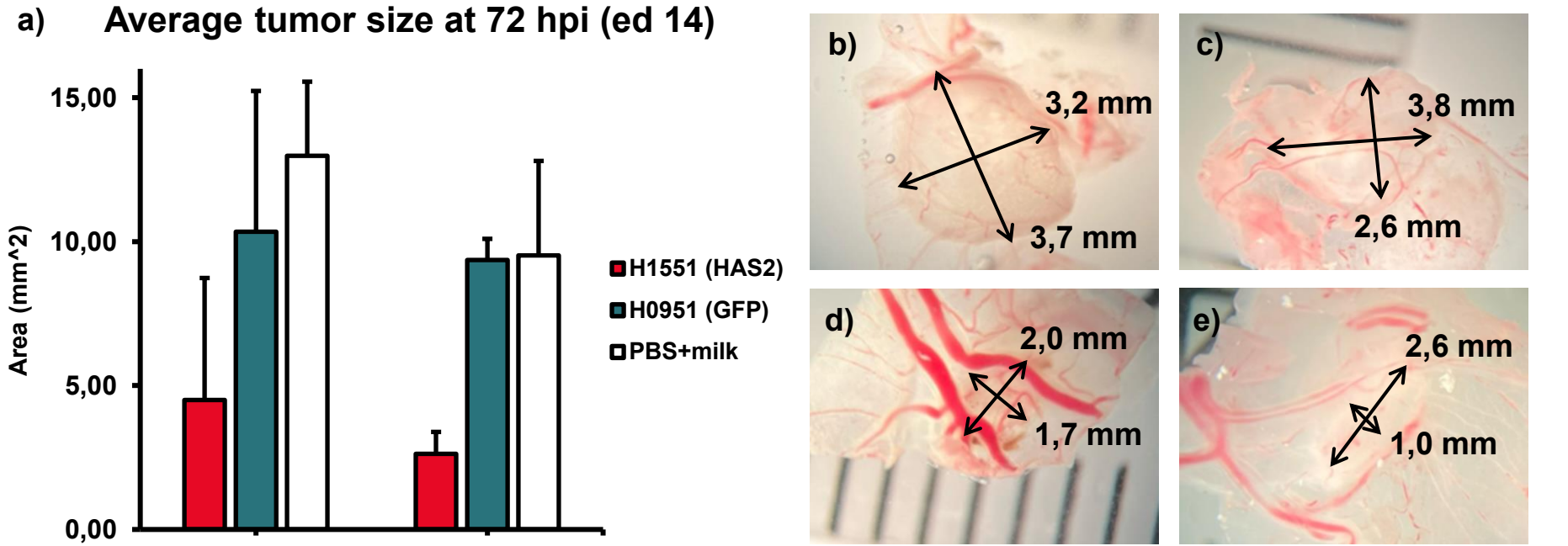


Figure 6: Average U373MG tumor size 72 hpi (a) (n = 2-6). Group 1 was seeded with 2 M cells and infected with 0,25 MPFU of virus, Group 2 was seeded with 0,5 M cells and infected with 0,1 MPFU of virus. Photos of an uninfected tumor (b), tumor infected with H0951 (*gfp*) (c) and tumors infected with H1551 (*has2*) (d, e).

Conclusion

- Our results support the observation that the *has2* gene variant XM_004838010 could have an anti-cancer effect in humans, provide an insight that the effect is mediated through the regulation of cell cycle and demonstrate that the effect can be induced in glioma cells with an HSV vector expressing the gene.

References

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