Ancient Intruders: Human Endogenous Retroviruses (HERVs) in Glioblastoma

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Introduction

Human Endogenous Retroviruses (HERVs):
- ancient retroviral sequences that integrated into the primates' genome millions of years ago
- account for about 8% of the human genome
- expressed in various human cancers
- over evolutionary time have become highly mutated
- mostly no longer encode functional genes

HERV-K:
- the most recently integrated HERV family
- contains intact open reading frames and expresses viral proteins

Glioblastoma Multiforme (GBM):
- one of the most aggressive human cancers
- current standard of care: surgery followed by radiation and Temozolomide (TMZ)
- limited treatment options, especially for recurrent tumors resistant to TMZ

Rationale:
- Expression from HERV loci is linked to cancer stemness and drug resistance.
- Deciphering connections between HERV-K and GBM development, progression, stemness, and drug resistance could prompt new therapeutic strategies.

Objectives:
- Characterize HERV-K expression in multiple human GBM cell lines compared to normal human astrocytes (NHA)
- Compare HERV-K expression in GBM cell lines sensitive and resistant to TMZ

Materials and Methods

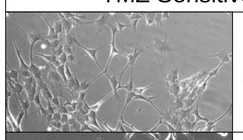
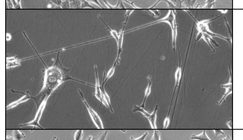
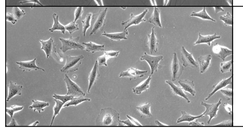
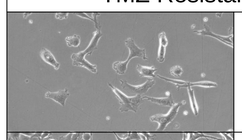
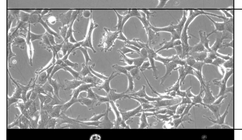
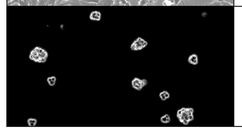
Image	Cell Line
TMZ Sensitive	
	Normal Human Astrocytes
	U87-MG
	LN229

Image	Cell Line
TMZ Resistant	
	U118-MG
	T98-G
	GBM-12 [Spheres]

RT-qPCR	Reverse Transcription Qualitative Polymerase Chain Reaction (RT-qPCR): detection and quantification of specific RNA
Western Blot	Detection of specific proteins
Immunofluorescence	Visualization of cell components with combinations of specific antibodies labeled with fluorophores

Results: RT-qPCR

- GBM cell lines express HERV-K RNA at lower levels than NHA.
- HERV-K expression varies among GBM cell lines.
- Differences in HERV-K RNA do not follow a consistent pattern between TMZ sensitive and resistant cells.

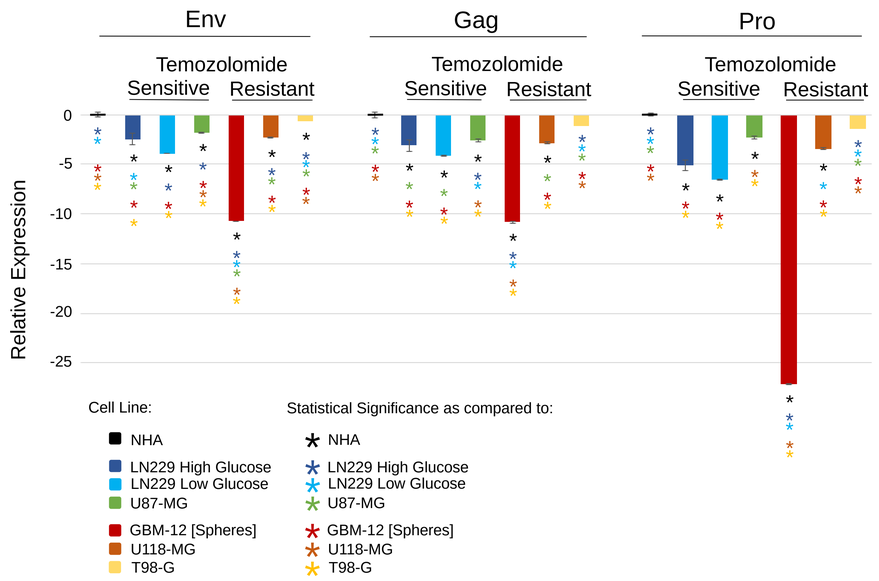


Figure 1. HERV-K RNA Expression Relative to NHA RT-qPCR results showing HERV-K RNA expression relative to NHA. Media glucose concentration: High: 4.5 g/L. Low: 1 g/L. Each value represents the mean±S.D. Statistical significance was determined by T-test [p-value < 0.05].

Results: Western Blot

- HERV-K Gag protein is detected in all cell lines and presents lower levels in GBM cell lines compared to NHA.
- HERV-K Env is not observed at the protein level.
- Media glucose concentration does not affect HERV-K Gag protein level.

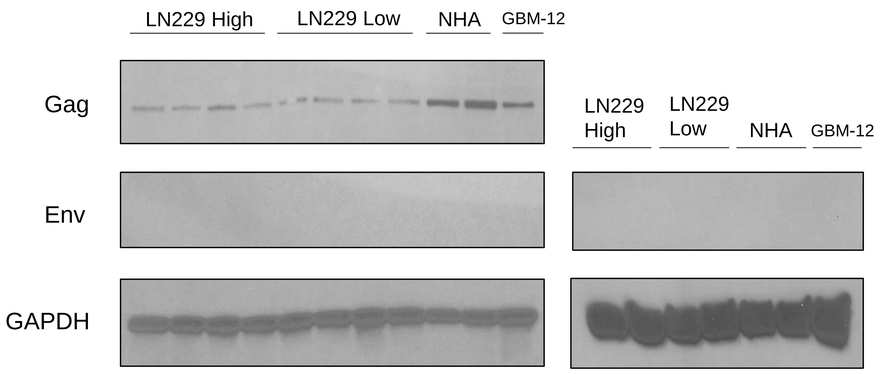


Figure 2. 2 Western Blots showing the expression of HERV-K proteins Gag and Env as well as GAPDH housekeeping gene as control. Media glucose concentration: High: 4.5 g/L. Low: 1 g/L.

Results: Immunofluorescence

- HERV-K Gag protein subcellular localization shows no differences between tested cell lines.

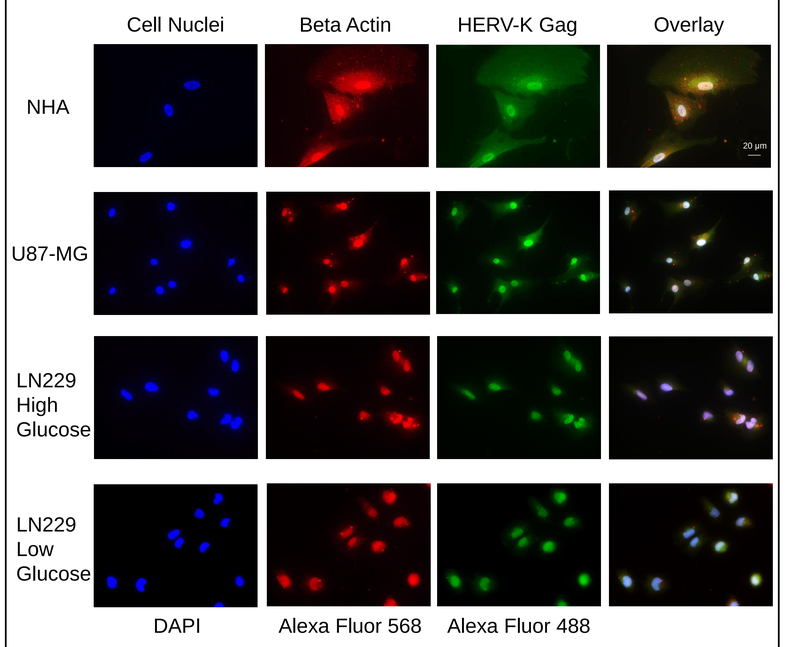


Figure 3. Immunocytochemical detection of HERV-K Gag protein in selected cell lines. Fluorescence microscopy images acquired by Keyence BZ-X microscope. Scale bar—20 µm. Media glucose concentration: High: 4.5 g/L. Low: 1 g/L.

Summary

- GBM cell lines show less HERV-K expression than NHA.
- Gag is observed at both the RNA and protein level.
- Env is present only at the RNA level.
- Media glucose level has no observable effect on HERV-K expression.
- HERV-K expression varies among GBM cell lines.
- Differences in HERV-K RNA do not follow a consistent pattern between TMZ sensitive and resistant cells.

Conclusions

- Our data indicates that HERV-K may be involved in GBM biology and suggests the potential use of HERV-K expression in diagnostic and/or therapeutic strategies.

Future Directions

- The contribution of HERV-K expression to cell phenotype will be further addressed with a CRISPR gRNA multiplexing method to induce or silence HERV-K expression.

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