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Upregulation of human endogenous retrovirus-K (HML-2) mRNAs in hepatoblastoma: Identification of potential new immunotherapeutic targets and biomarkers☆

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ABSTRACT

Purpose: Hepatoblastoma is the most common liver malignancy in children. In order to advance therapy against hepatoblastoma, novel immunologic targets and biomarkers are needed. Our purpose in this investigation is to examine hepatoblastoma transcriptomes for the expression of a class of genomic elements known as Human Endogenous Retrovirus (HERVs). HERVs are abundant in the human genome and are biologically active elements that have been associated with multiple malignancies and proposed as immunologic targets in a subset of tumors. A sub-family of HERVs, HERV-K(HML-2) (HERV-K), have been shown to be tightly regulated in fetal development, making investigation of these elements in pediatric tumors paramount.

Methods: We first created a HERVK-FASTA file utilizing 91 previously described HML-2 proviruses. We then concatenated the file onto the GRCh38.95 cDNA library from Ensembl. We used this reference database to evaluate existing RNA-seq data from 10 hepatoblastoma tumors and 3 normal liver controls (GEO accession ID: GSE8977575). Quantification and differential proviral expression analysis between hepatoblastoma and normal liver controls was performed using the pseudo-alignment program Salmon and DESeq2, respectively.

Results: HERV-K mRNA was expressed in hepatoblastoma from multiple proviral loci. All expressed HERV-K proviral loci were upregulated in hepatoblastoma compared to normal liver controls. Five HERV-K proviruses (1q21.3, 3q27.2, 7q22.2, 12q24.33 and 17p13.1) were significantly differentially expressed (p-adjusted value <0.05, $|\log2 \text{ fold change}| > 1.5$) across conditions. The provirus at 17p13.1 had an approximately 300-fold increased expression in hepatoblastoma as compared to normal liver. This was in part due to the near absence of HERV-K mRNA at the 17p13.1 locus in fully differentiated liver samples.

Conclusions: Our investigation demonstrates that HERV-K is expressed from multiple loci in hepatoblastoma and that the expression is increased for several proviruses compared to normal liver controls. Our results suggest that HERV-K mRNA expression may be useful as a biomarker in hepatoblastoma, given the large differential expression profiles in hepatoblastoma, with very low mRNA levels in liver control samples.

Hepatoblastoma is the most common pediatric liver malignancy, affecting approximately 500 children in the US each year [1,2]. Similar to other fetal tumors, hepatoblastoma is thought to arise from embryonic liver progenitor cells that fail to differentiate into hepatocytes [3–5]. As hepatoblastoma precursor cells show different levels of differentiation prior to malignant transformation, this cancer is morphologically complex and histologically subcategorized as one of the following subtypes: fetal, embryonal, or mixed epithelial and mesenchymal undifferentiated small cell [6]. Treatment is multimodal, involving a combination of resection and chemotherapy or transplant [7]. Five-year survival in North America is between 70 and 80%, with the best outcomes in early stage disease [8,9]. There is still a clear need to identify novel treatment strategies that can offer more children

Abbreviations: HERV-K, Human Endogenous Retrovirus-K; HB, Hepatoblastoma; FT, Fetal Tumor; NC, Normal Liver Control.

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hope for a long-term cure [10,11]. A full understanding of the molecular drivers of these tumors will be advantageous in the search for new treatments [12].

This report focuses on the expression of Human Endogenous Retrovirus-K (HML-2) (HERV-K) mRNA in hepatoblastoma. Human endogenous retroviruses (HERVs) are transposable genomic elements that have integrated into the human germline over many millions of years. Altogether, HERVs comprise an estimated 8% of the human genome [13]. For in-depth reviews of HERV biology, see [14–16]. HERV-K viruses represent the most recent retroviruses to integrate into the human germline and many copies are still capable of producing viral proteins [17–19]. Interestingly, when expressed, HERV-K viral proteins are capable of activating both innate, humoral and cell-mediated immune responses [20–24].

Though often transcriptionally silent in normal cells, HERV-K mRNA expression has been demonstrated in multiple cancers including hepatocellular carcinoma [25–27]. Given that HERV-Ks often demonstrate large differences in expression profiles between cancer and non-cancer tissue, as well as their ability to activate the immune system, attention has recently turned to the potential role of HERVs as both targets for immunotherapy [28–31] as well as tumor markers in disease [32–34]. For the current understanding of HERV-K biology in cancer, see [35,36].

HERV-K mRNA expression has also been demonstrated during embryogenesis, but is progressively silenced as fetal development continues [37,38]. Transcriptional activity of HERV-K proviruses in pediatric tumors are thus of specific interest as these cancers are thought to arise from embryonic precursor cells that fail to differentiate during organ development. We sought to investigate HERV-K expression in hepatoblastoma to explore the hypothesis that HERV-K mRNA may be more expressed in hepatoblastoma compared to fully differentiated, non-cancer liver tissue. Furthermore, investigation of these elements may lead to the identification of novel tumor markers or new immunotherapeutic targets for the treatment of hepatoblastoma.

1. Methods

1.1. Hepatoblastoma and normal liver RNA-seq data

The dataset used in this investigation includes RNA sequencing (RNA-seq) data from 10 clinically aggressive hepatoblastoma samples and 3 normal liver controls taken from non-malignant adult liver tissue prior to transplantation. Tumor excision and RNA isolation was performed by the University of Pittsburg Children's Hospital as part of a next-generation sequencing (NGS) study to identify activated cancer pathways in clinically aggressive hepatoblastoma [39]. The raw sequencing data are publicly available and were downloaded from the NCBI biorepository using the NCBI Sequence Read Archive (SRA) Toolkit (GEO accession ID GSE89775). According to the NCBI biorepository, total RNA (1 μg) was isolated from fresh frozen tissue (both hepatoblastoma and normal liver) and sequenced on an Illumina platform to generate 100 base-pair, strand-specific, paired-end reads to a sequencing depth of approximately 40 M reads per sample. Prior to analysis in this study, all raw FASTQ files were pre-processed with Trimmomatic to remove adaptors and low-quality reads as well as to assure that only paired-ends reads with a minimum read length of 50 nucleotides were included [40]. The quality of the raw and trimmed reads from each sample was confirmed with the program FASTQC (http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc).

1.2. Analysis of HERV-K mRNA expression

HERV-K proviruses are currently not well annotated in the human genome and thus standard RNA-seq analysis techniques cannot be used to determine the expression profile of HERV-Ks. To overcome this limitation, we created a HERV-K specific FASTA file using the genomic sequence of the 91 HERV-K proviruses deposited in NCBI (GenBank ID JN675007-JN675097) [19]. To determine the transcriptional profile and differential expression of HERV-K in all samples, we concatenated our working HERV-K FASTA file onto the GRCh38.95 cDNA fasta file downloaded from Ensembl. For RNA analysis, we did not annotate the individual potential spliced transcripts that would be expected to be expressed from an integrated provirus, but rather defined the entire proviral sequence as a single transcript. We then used the pseudo-aligner, Salmon [41] in mapping-based mode with the validateMappings flag to create a count matrix over the full human transcriptome including the concatenated HERV-K file (example code: salmon quant -i GRCh38_HERVK.fa -l A -1 FT6_1.fq −2 FT6_2.fq –validateMappings -o FT6_quant). We then sub-selected read counts assigned to HERV-K loci.

1.3. HERV-K expression profiles and differential expression

Transcript abundance read estimates from Salmon were imported into R (version 3.5.1) using tximport [42]. Transcript abundance estimates were normalized for sample sequencing depth using the R Bioconductor package DESeq2 [43]. This allowed us to determine normalized HERV-K expression across all proviral loci by sample, together with the number of loci responsible for total HERV-K expression and the range of reads across each locus. Differential expression of HERV-K in hepatoblastoma as compared to normal liver was also analyzed using DESeq2. HERV-K proviruses were considered differentially expressed if the p-adjusted values (calculated using the Benjamini-Hochberg False Discovery Rate implemented in DESeq2) were less than 0.05 and the absolute value of the log2 fold changes were greater than 1.5 [44].

Given the apparent heterogeneity in HERV-K expression across different hepatoblastoma samples, we also stratified hepatoblastoma samples by overall HERV-K expression (total number of normalized reads across all proviral loci). We selected the top 3 highest HERV-K expressing hepatoblastoma samples and the three lowest HERV-K expressing hepatoblastoma samples. We then performed a differential gene expression analysis again using DESeq2 to compare the high expressing to low expressing tumors. Genes with a p-adj value <0.05 and $|\log2 \text{ fold change}| > 1.5$ were considered significant and included in a Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) over-representation functional analysis. Gene pathways were considered enriched if they had a p-adj value <0.05. GO and KEGG analysis was performed using the clusterProfiler package in R [45].

Unless otherwise specified, all plots denoting the RNA expression profile and differential expression were generated using the ggplot2 package in R [46]. The scatterplot was created using the EnchancedVolcano visualization package in R [47].

2. Results

The RNA-seq dataset that was downloaded from the NCBI biorepository included 10 hepatoblastoma samples (HB) from children with unresectable disease undergoing liver transplantation. These samples represent aggressive hepatoblastomas that were not amenable to up-front resection, and all children received appropriate neo-adjuvant chemotherapy prior to surgery [48]. It also included 3 normal liver controls (NC) from orthotopic adult livers prior to transplant. Following pseudoalignment with Salmon and sample normalization with DESeq2, we found that the HERV-K RNA expression profile varied greatly across the hepatoblastoma (HB) samples and normal liver controls (NC). In HB, the median HERV-K read counts across all proviral locations for each sample was 342 (interquartile range (IQR) 235, 515). However, 2 samples had greater than 2000 reads that aligned to several HERV-K proviruses, whereas 2 samples showed 150 read counts or less (Table 1). The general HERV-K expression profile across all samples is visualized in the HERV-K expression heatmap in Fig. 1. Each cell in the heatmap represents the normalized expression Z-score calculated across samples (range − 3 to 3) of a specific HERV-K provirus (y-axis) in individual HB or NC

Table 1
Normalized HERV-K read counts and number of transcribed HERV-K loci in hepatoblastoma and normal liver controls.

Sample	Normalized read count across all HERV-K Loci	Total HERV-K locations with >10 normalized reads	Range of reads across individual provirus
Hepatoblastoma			
FT6	273.2	10	10.4–29.5
FT7	223.3	5	12.6–74.6
FT8	532.0	3	20.1–363.8
FT9	2778.8	42	14.4–243.5
FT10	148.3	1	10.1
FT11	2503.8	28	22.2–410.3
FT12	270.4	9	10.4–26.9
FT13	465.1	13	10.5–162.1
FT14	412.2	12	10.2–51.2
FT15	153.0	2	11.9–35.1
Normal liver			
NC1	141.2	3	11.5–50.3
NC2	98.1	1	14.6
NC3	203.4	7	11.6–23.4

Abbreviations: FT, fetal tumor (hepatoblastoma); NC, normal control (normal liver).

samples (x-axis). mRNA from numerous proviral loci were expressed at average levels across all the samples (Z-score = 0), which we confirmed were HERV-Ks expressed at low levels (average of less than 10 reads across a HERV-K provirus in individual samples). These HERV-K loci are represented by light blue in the heatmap. There was great heterogeneity in proviral expression in individual samples, including variation among the hepatoblastoma samples (9.5 expressed proviral loci, IQR 9.3). Variation was also observed in the normal liver controls (3 expressed proviral loci, IQR 3) although the total number of expressed HERV-K loci was less than in HB. No proviral loci were common to all samples and many HERV-K loci were expressed in less than 5 total samples.

We next calculated differential proviral expression across the HB and NC samples. All expressed proviral loci were upregulated in HB compared to NC as demonstrated by the HERV-K expression profile scatter plot (Fig. 2). Five proviruses (1q21.3, 3q27.2, 7q22.2, 12q24.33 and 17p13.1) were significantly differentially expressed (p-adjusted value <0.05, |log2 fold change| > 1.5) across conditions (Table 2). For these five proviruses, boxplots of the log10 normalized counts for the HB and NC samples across each differentially expressed provirus reveal much higher expression in HB compared to NC samples (Fig. 3), although for 3 proviruses (17p13.1, 3q27.2 and 7q22.2), the normalized expression across all 3 NC samples was less than 10 reads and was too low for graphical comparison. The absence of expression in NC led to large fold-changes between HB and NC. Thus, 17p13.1 showed a 294-fold increase expression in HB as compared to NC (padj = 0.009). This provirus was expressed in all hepatoblastoma samples with one exception and was not expressed in 2 of the 3 normal controls. Similarly, in HB samples, 7q22.2 was expressed 93.1-fold above NC (padj = 0.027), while 3q27.2 was expressed 55-fold above NC (padj = 0.026).

Hepatoblastoma samples FT8 (531 HERV-K reads), FT9 (2778 HERV-K reads and FT11 (2503 HERV-K reads) represented the tumor samples with the highest HERV-K expression. Conversely, FT7 (223 HERV-K reads), FT10 (148 HERV-K reads) and FT15 (152 HERV-K reads) represented the tumors with the lowest expression. A differential expression analysis comparing the high to low tumors revealed 775 differential expressed genes. GO Biological Process enrichment analysis of the differentially expressed genes revealed over-representation of cellular processes involved in leukocyte activation and leukocyte mediated immunity (Table 3). Furthermore, global cell processes including immune effector processes and cell activation involved in immune responses

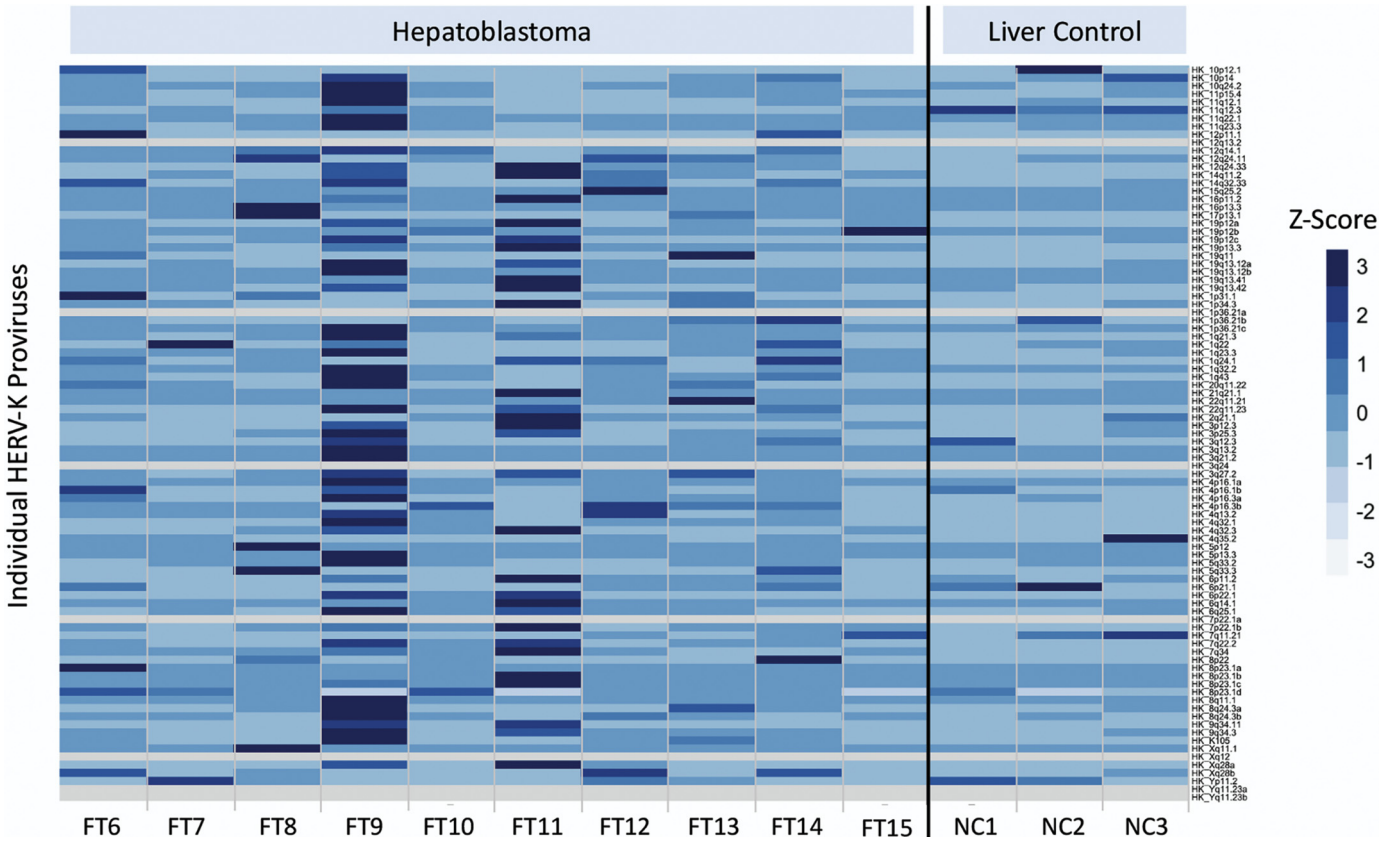

Fig. 1. Heatmap of HERV-K expression in hepatoblastoma and normal liver controls. All tumor samples and normal liver controls are represented on the x-axis. All HERV-K proviral loci are represented on the y-axis. Color expression key is located in upper left of figure and is based on the calculated Z score (scale −3 to 3) across all samples in the study. Each individual cell represents the normalized HERV-K proviral expression (x-axis) in the respective sample (y-axis).

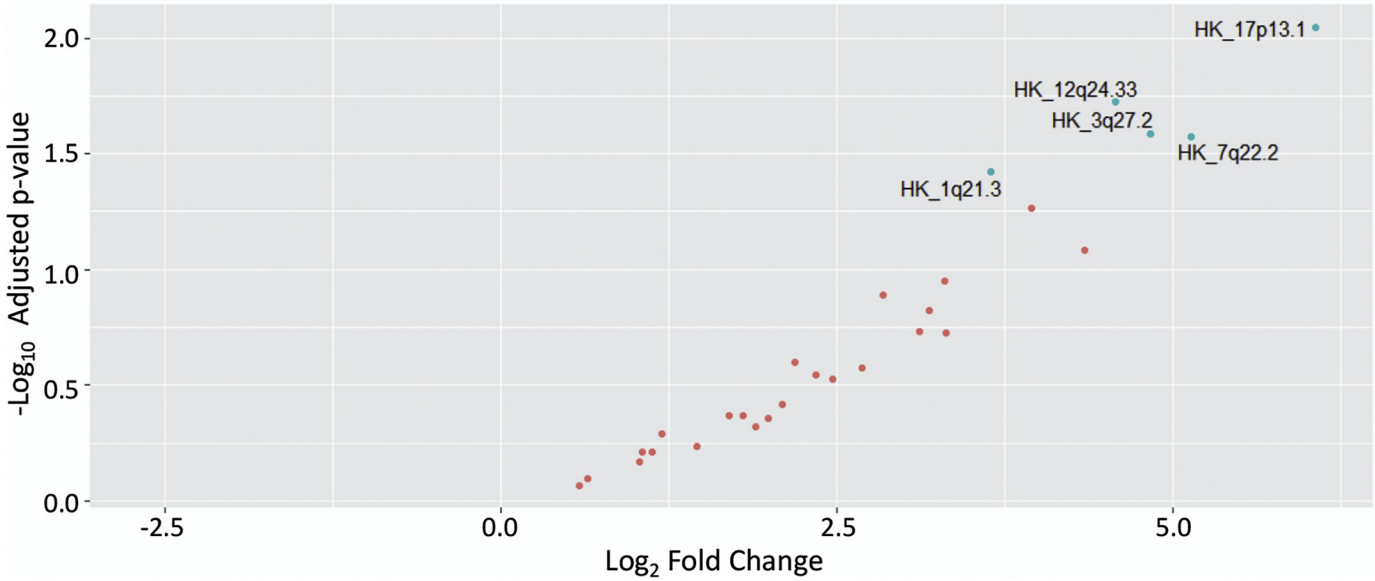

Fig. 2. Scatter plot of HERV-K differential expression profile between hepatoblastoma and normal liver controls. Each point on the figure represents the log2 fold change (x-axis) between conditions of an individual HERV-K provirus plotted against the corresponding -log10 p-adjusted value (y-axis). Orange points represent HERV-K proviruses that were not significantly differentially expressed (p-adj < 0.05, |log2fold change| > 1.5) between conditions. Green points represent differentially expressed provirus, which are also labeled by genomic location (HK_1q21.3 represents the HERV-K provirus located at 1q21.3).

were significantly enriched in hepatoblastoma samples that demonstrated increased HERV-K expression patterns.

3. Discussion

The data in this investigation establish that several HERV-K proviruses are expressed in hepatoblastoma. Furthermore, the mRNA profile of HERV-K in hepatoblastoma is complex, with multiple proviruses transcribed from different loci in tumors from different individuals. The data also show that overall HERV-K expression is increased in hepatoblastoma compared to normal liver controls and that several proviruses show large fold increases in tumors compared to normal liver tissue. The significant increase makes HERV-Ks intriguing targets for immunotherapy. In addition, our data suggest that they could also serve as potential biomarkers for disease recurrence or progression, though further studies are required to confirm this. This investigation is the first to demonstrate HERV-K expression in a pediatric solid organ malignancy. In addition, the bioinformatics pipeline described in this manuscript provides an effective tool to measure HERV-K RNA profiles in disease versus non-disease states that could be used to screen other fetal malignancies for HERV-K expression.

3.1. Expression of HERV-K in hepatoblastoma

Multiple HERV-K proviruses showed increased expression in hepatoblastoma. The HERV-K provirus at 17p13.1 was the most dramatic example of a large differential expression value, with an almost 300-fold change in expression from the provirus compared to normal liver tissue. Similarly, large differential expression values were seen for proviral loci 1p21.3, 3q27.2, 7q22.2 and 12q24.33.

The magnitude of the increased expression levels over normal liver controls was prominent in several instances. This is in part because mRNA levels from HERV-K proviruses in normal liver control were either not present at all, or present at very low levels (less than 10 read counts across the entire provirus). Our findings of low HERV-K expression in fully differentiated liver is consistent with previous investigations that have examined HERV-K expression in liver tissue [35,49]. This finding is also consistent with the reported low levels of HERV-K expression in the majority of fully differentiated somatic tissues [35,49].

Proviral expression profiles also differed across the hepatoblastomas themselves. Several hepatoblastoma samples had over 100 read counts aligned to the provirus at 17p13.1, while several other tumors had less than 10 counts (which was more similar to the expression profile in NC). This variation in proviral expression across HB samples was true for total HERV-K expression as well. Two hepatoblastoma samples had over 2000 normalized read counts summed over all proviruses. In contrast, two HB samples had ~150 normalized read counts across all proviruses with only 1 or 2 proviral locations with over 10 read counts. The HERV-K RNA expression in these tumors was thus more similar to the normal liver controls than to the other tumor samples. In follow-up investigations it will be important to determine whether HERV-K expression correlates with the molecular subtype of hepatoblastoma.

Table 2
Differential gene expression of HERV-K between hepatoblastoma and normal liver controls.

HERVK provirus	Log2 fold change	Log2 fold change standard error	p-Value	p-Adjusted value
1q21.3	4.0397	1.3917	0.00369	0.03891
3q27.2	5.8226	1.8781	0.00193	0.02645
7q22.2	6.5405	2.1196	0.00203	0.02713
12q24.33	5.3952	1.6643	0.00119	0.01941
17p13.1	8.2095	2.3046	0.00037	0.00927

3.2. Potential clinical applications of HERV-K in hepatoblastoma

The extremely large fold changes found in HERV-K expression across hepatoblastoma and non-cancer tissue make these genomic elements prime targets as tumor specific antigens, which has been well described in multiple cancers [50–54]. Establishing HERV-K proviruses as a tumor specific antigen in hepatoblastoma leads to intriguing follow up questions, specifically, if these elements may function as novel tumor markers for clinical subtypes of hepatoblastoma or if these elements may act as neoantigens and present novel targets for immunotherapy.

Our current RNA-seq investigation addressed HERV-K expression in tumor verses non-tumor tissue samples and did not address HERV-K expression profiles in peripheral blood samples from patients, limiting our ability to directly address feasibility as a tumor marker. Though we were

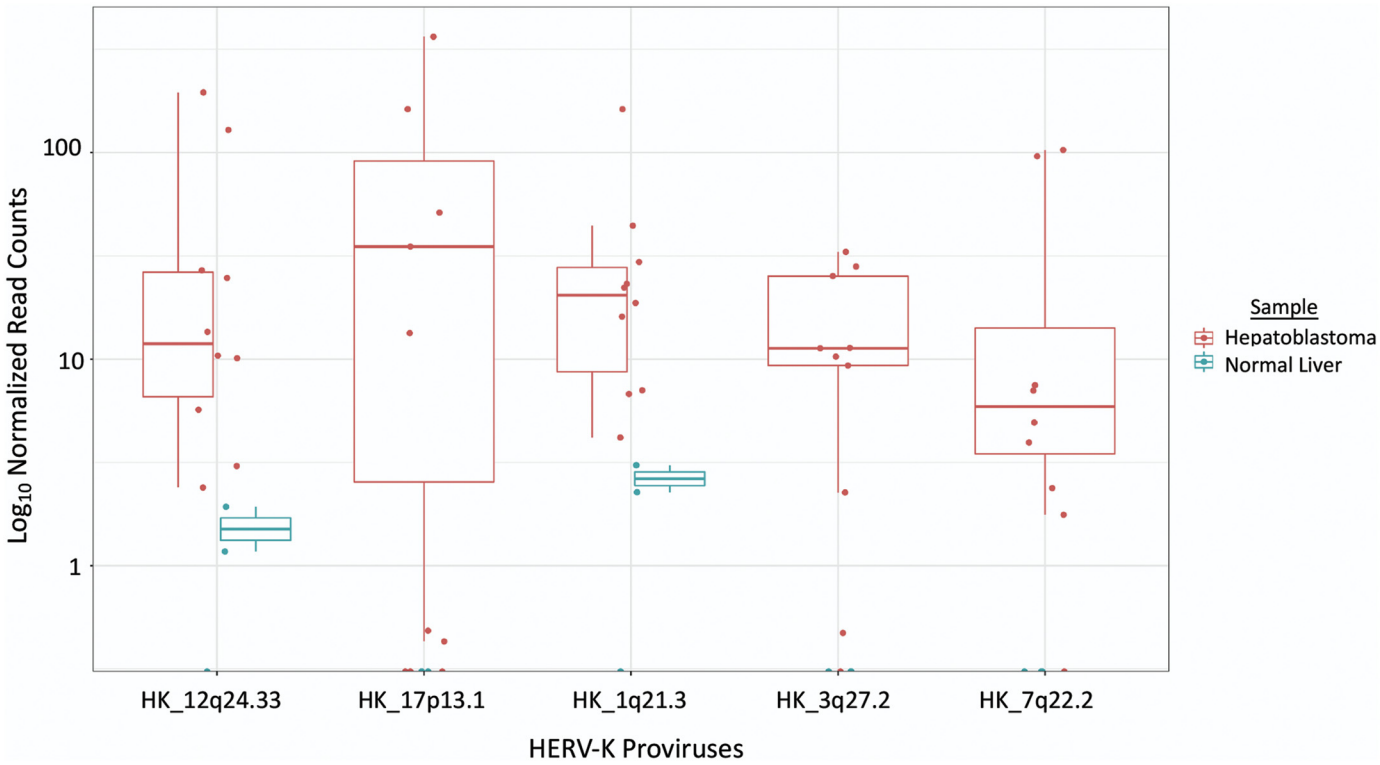

Fig. 3. Boxplot and overlaid dotplot of significantly differentially expressed HERV-K proviruses. Individual proviruses are represented on the x-axis. Log10 normalized count values are represented on the y-axis. The normalized count value for each provirus in each sample is represented by an individual colored point. Hepatoblastoma samples are represented in orange while normal liver controls are represented in green. The central line of the boxplot is determined by the median log10 normalized expression value across all grouped samples (hepatoblastoma or normal liver controls) for each significantly differentially expressed provirus. The 'box' represents the 25th (lower line) and 75th (upper line) percentile log10 normalized expression across grouped samples.

unable to evaluate this in our current investigation, increased HERV-K expression has been demonstrated in both breast cancer tumors as well as the serum of breast cancer patients [55]. In addition, HERV-K expression can effectively differentiate basal cell carcinoma from other breast cancer subtypes [54]. The data in this investigation, combined with studies on other tumors, strongly support further investigation of HERV-K expression in peripheral bloods samples of hepatoblastoma patients to determine if HERV-K expression can be used as an effective tumor marker for disease reoccurrence or treatment resistance.

Regarding HERV-K expression as a potential neoantigen for immunotherapy, a differential gene expression analysis of high HERV-K expressing tumors versus low HERV-K expressing tumors provided promising results. A GO enrichment analysis of the differentially expressed genes demonstrated a strong correlation with cellular pathways involving leukocyte activation as well as neutrophil and leukocyte mediated immunity. Our data suggest that HERV-K mRNA levels may correlate with either direct tumor immunogenicity or an inflammatory microenvironment surrounding the tumors. The possibility that

Table 3
Gene Ontology Biological Process Enrichment Analysis following differential gene expression analysis of high HERV-K expressing HB vs low HERV-K expressing HB.

Functional category	Differentially expressed genes	Total genes in functional pathway	Enrichment False discovery rate (adjusted p-value)
Regulated exocytosis	82	901	1.33E-12
Exocytosis	85	1023	2.41E-11
Neutrophil activation	61	594	2.41E-11
Granulocyte activation	61	603	2.55E-11
Myeloid leukocyte activation	69	766	8.96E-11
Myeloid leukocyte mediated immunity	62	647	8.96E-11
Neutrophil mediated immunity	59	591	8.96E-11
Secretion	124	1861	8.96E-11
Myeloid cell activation involved in immune response	61	640	1.60E-10
Neutrophil activation involved in immune response	57	583	3.11E-10
Cell activation	109	1591	4.16E-10
Neutrophil degranulation	56	577	5.59E-10
Secretion by cell	114	1715	6.51E-10
Leukocyte degranulation	59	632	6.51E-10
Vesicle-mediated transport	134	2220	5.32E-09
Immune effector process	96	1392	5.32E-09
Leukocyte mediated immunity	74	965	1.11E-08
Leukocyte activation	96	1416	1.21E-08
Cell activation involved in immune response	66	819	1.48E-08
Leukocyte activation involved in immune response	65	815	3.06E-08

* Table represents the top 20 functional categories in the Gene Ontology Biological Process Enrichment Analysis stratified by false discovery rate.

expressed HERV-K proteins in these tumors could be acting as cancer neoantigens capable of activating an immune response is thus an intriguing possibility suggested by our data. It is possible for any of the 5 identified proviruses that are differentially expressed to act as triggers for either the innate or adaptive immune system and produce protein epitopes capable of acting as neoantigens. However, the provirus at 17p13.1, 12q24.33 and 1q21.3 are more likely to represent appropriate targets given that they have expression across viral proteins [56].

HERV-K expression correlating with tumor immunogenicity is supported by recent literature. Rooney et al. analyzed approximately 20 solid organ tumors as well as normal tissue controls from TCGA mRNA-seq datasets for expression of both endogenous retrovirus (ERV) families as well as cytolytic activity. The investigation found that several tumor specific ERVs existed across multiple tumors and that high expression of tumor-specific ERVs significantly correlated with immune activation [57]. In regards to immunotherapy, chimeric antigen receptor (CAR) T cells that target HERV-K Env proteins have been developed and tested in in vivo murine models for both breast cancer and melanoma [30,31]. In both models, the HERV-K Env CAR T-cells demonstrated tumor specific cytotoxicity, reduced the primary tumor mass and showed reduction of tumor metastases.

One of the limitations of our investigation is that all tumors samples represented advanced disease (unresectable hepatoblastoma require transplantation) and all tumors were exposed to chemotherapy prior to RNA isolation. Little data exists which directly investigates the effect of cytotoxic chemotherapy on HERV-K expression. However, one study in breast cancer suggests that cytotoxic chemotherapy reduced HERV-K expression in peripheral blood samples [55]. Though it is difficult to extrapolate to hepatoblastoma, it may be that neo-adjuvant chemotherapy reduced HERV-K expression in some of our hepatoblastoma samples. A key future investigation will be to evaluate HERV-K expression in early-stage, treatment naïve tumors, which may demonstrate more robust findings.

In our differential expression and gene enrichment analysis we did not find any correlation with cell differentiation or activated cancer pathways to directly suggest a role in embryonal tumorigenesis. Previous literature suggests that HERV-K proviral enhancers can affect transcription of cellular genes up to 100,000 base pairs upstream or downstream of the provirus [58]. Additionally, HERV-K-*env* expression has specifically been linked to perturbations in the TP53 signaling pathways in breast cancer [26]. Though we saw strong expression from the HERV-K provirus at 17p13.1, we did not see alterations in the transcriptional expression of TP53 in our data. It remains unclear, as it does for many HERV investigations, whether HERV-K expression acts as a disease driver in hepatoblastoma or is a result of global epigenetic changes in the cancer cell [59]. What is clear from the current investigation is the tumor specificity and the tumor immunogenicity of HERV-K expression in hepatoblastoma.

3.3. Overall limitations of this study

There are several limitations in the dataset that we used in this analysis. The use of non-patient matched liver control tissue prevented a more thorough analysis of differential expression between tumors and normal liver, given that HERV-Ks remain polymorphic in the human population. A lack of aged-matched and patient-matched normal tissue controls is a common issue with current RNA-seq analysis studies of fetal solid organ malignancies. Additionally, as highlighted in the discussion, all tumors samples were subjected to neoadjuvant cytotoxic chemotherapy. It is impossible to predict how this exposure directly affected HERV-K expression in hepatoblastoma, though it potentially could have suppressed expression. The small dataset did not allow the ability to correlate HERV-K transcription with demographic data including gender and age or clinical outcomes, including stage of disease, reoccurrence, disease resistance and over-all survival. It also limited the potential to correlate HERV-K expression with differentiation/histologic subtypes of hepatoblastoma including fetal, embryonal and undifferentiated disease. These will be important future investigations given the preliminary findings in this investigation that HERV-K expression appears to be highly tumor specific. Lastly, from a bioinformatics perspective, a future annotation of all viral mRNAs – including Gag, Env, Rec and/or Np9 that can be produced from each individual provirus, will allow for direct screening of viral mRNAs that have the potential to encode for viral proteins.

4. Conclusion

The current investigation demonstrates that several Human Endogenous Retrovirus-K proviruses are transcribed in hepatoblastoma, with increased RNA expression from several proviral loci in hepatoblastoma as compared to normal liver controls. The large difference in HERV-K expression profiles between hepatoblastoma and normal liver make HERV-K expression an intriguing target as a tumor specific antigen. Future investigations are required to explore HERV-K expression as a tool for molecular disease stratification, as well as for targeted immunotherapy. Finally, our study highlights the important need to continue to develop tumor banks for pediatric solid organ tumors that include patient matched tissue controls and treatment-naïve tissue samples for appropriate molecular comparison.

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