Expression of Human Endogenous Retroviruses (HERVs) in Breast Cancer: A Transcriptomic Study

Dimitra Bartzi *, Anastassios Philippou, Ioanna Tsatsou, Michael Koutsilieris

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Article

Expression of Human Endogenous Retroviruses (HERVs) in Breast Cancer: A Transcriptomic Study

Dimitra Bartzi [1,*], Anastassios Philippou [2], Ioanna Tsatsou [3] and Michael Koutsilieris [4]

[1] Oncology Resident, Hellenic Airforce General Hospital & Researcher, Medical School, University of Crete, Herakleion, & MSc in Molecular and Applied Physiology, Medical School, National & Kapodistrian University of Athens, Greece. demmy345@hotmail.com

[2] Medical School, National and Kapodistrian University of Athens, Athens, Greece. tfilipou@med.uoa.gr

[3] Cancer and Palliative Care, Oncology Department, Hellenic Airforce General Hospital, Athens, Greece. itsatsou@uniwa.gr

[4] Endocrinologist, Professor of Physiology, National & Kapodistrian University of Athens, Athens, Greece. mkoutsil@med.uoa.gr

* Correspondence: demmy345@hotmail.com

Abstract: Background: Human endogenous retroviruses (HERVs) are ancient viral elements integrated into the human genome, accounting for approximately 8% of its content. Recent findings suggest that HERVs, especially HERV-K (HML-2), play a significant role in the onset and advancement of breast cancer. Understanding HERV expression patterns could reveal new biomarkers and therapeutic targets in breast cancer. This study examined the expression of endogenous retroviruses in human breast tissues, to reveal potential associations between retroviruses and breast cancer. **Methods:** RNA-Seq datasets from breast cancer tissues (ductal carcinoma and triple-negative subtypes) and normal breast tissues were retrieved from the NCBI Sequence Read Archive (SRA). Bioinformatics analysis included read mapping against the human genome (hg19) and a custom pseudogenome containing HERV sequences. Normalized expression of HERV-human junctions was calculated and statistical comparisons were performed using independent samples t-tests and Mann-Whitney tests**. Results:** Breast cancer tissues exhibited significantly higher normalized coverage of HERV integration sites compared to healthy controls ($p=0.009$). Among breast cancer cases, patients who had undergone treatment showed significantly lower HERV expression than untreated patients ($p=0.023$). Visualization via IGV confirmed increased read accumulation at HERV loci in cancer samples. **Conclusion:** This study showed that HERVs are overexpressed in breast cancer tissues compared to normal breast tissues, with expression levels influenced by therapeutic interventions. These findings support the potential role of HERVs as biomarkers for breast cancer diagnosis, prognosis, and therapeutic monitoring. Further research is warranted to explore HERV-targeted strategies for breast cancer treatment.

Keywords: HERVs; breast cancer; transcriptomics; RNA-Seq; biomarkers; gene expression; therapy response

1. Introduction

Breast cancer remains a leading cause of cancer-related mortality among women globally [1]. In 2024, it is estimated that approximately 310.720 new cases of invasive breast cancer were diagnosed in women in the United States, along with about 56.500 cases of ductal carcinoma in situ (DCIS). Breast cancer continues to account for around 30% of all new cancer diagnoses in U.S. women. Mortality remains significant, with an expected 42.250 female deaths and 530 male deaths out of an estimated 2.790 new male cases. Despite these numbers, the five-year relative survival rate for breast cancer diagnosed at a localized stage exceeds 99%. This rate declines to 87% for regional-stage disease

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and drops further to 32% when diagnosed at a distant, metastatic stage. Encouragingly, breast cancer mortality has declined by 44% since 1989, resulting in over 517.900 averted deaths. However, concerning trends include a rising incidence among younger women under 50 years, increasing at an annual rate of 1.4% between 2012 and 2021 [2]. Significant racial disparities also persist, with Black women experiencing a 40% higher mortality rate compared to White women, largely due to later-stage diagnoses and unequal access to quality care [3].

Tumorgenesis is a multistep process, with oncogenic mutations in a normal cell conferring clonal advantage as the initial event. However, despite pervasive somatic mutations and clonal expansion in normal tissues, their transformation into cancer remains a rare event, indicating the presence of additional driver events for progression to an irreversible, highly heterogeneous, and invasive lesion [4]. Advances in molecular biology and genomics have revolutionized our understanding of tumor biology, yet many mechanisms contributing to breast cancer onset and progression remain poorly understood. Researchers are focusing on encompassing diverse aspects such as tumor stemness, intra-tumoral microbiota, and circadian rhythms [5]. Among the genomic elements receiving increasing attention are endogenous retroelements—particularly human endogenous retroviruses (HERVs) [6].

HERVs are sequences derived from ancient retroviral infections that have been integrated into the human genome over millions of years. Although most are defective and transcriptionally silent due to mutations and epigenetic repression, certain HERV families, especially human endogenous retrovirus type K [HERV-K (HML-2)], retain the ability to be transcribed under specific physiological and pathological conditions. In cancer biology, aberrant reactivation of HERVs has been reported in several malignancies, including melanoma, testicular cancer, and breast cancer [6–8].

The HERV-K (HML-2) subgroup is the most biologically active and has been implicated in oncogenesis through mechanisms such as insertional mutagenesis, induction of inflammation, and modulation of the immune microenvironment. The transcriptional activity of HERVs in tumor tissues may provide insights into novel oncogenic pathways and serve as a source of tumor-specific antigens [10,11]. The HERV-K (HML-2) is found to be expressed in various subtypes of breast cancer. This retrovirus was integrated into the primate genome approximately 55 million years ago, presenting a challenge for potential immunotherapy approaches for breast cancer. Recent studies have indicated that several endogenous retroviruses (ERVs) have been re-activated in tumors, with many exhibiting overexpression in cancerous tissues while showing low or undetectable levels in normal tissues [11,12].

This study focuses on evaluating the differential expression of HERVs in breast cancer versus normal breast tissue using publicly available RNA-Seq datasets. We also assessed the effect of treatment on HERV expression levels, with the ultimate goal of exploring HERVs as potential biomarkers and therapeutic targets in breast cancer.

2. Materials and Methods

2.1. Dataset Acquisition

RNA-Seq datasets were obtained from the NCBI Sequence Read Archive (SRA) [13]. All data has been securely downloaded into password-protected directories of the SRA. The SRA Toolkit was obtained through Linux to facilitate access to SRA data. This toolkit enables the creation of next-generation sequencing files in the specified format and cloud storage.

RNA-Seq data for breast ductal carcinoma (BAM files) along with their corresponding clinical information were sourced from the NCBI SRA. The paired-end FASTQ files for each sample were downloaded in SRA format and subsequently converted to FASTQ format using the fastq-dump command from the SRA Toolkit. Samples included 11 breast cancer tissues (various subtypes including ductal carcinoma and triple-negative) and 6 healthy breast tissues.

 doi:10.20944/preprints202505.1844.v1

2.2. Bioinformatics Analysis

The paired-end reads in FASTQ format were aligned to the human reference genome, hg19 using bowtie2 with default settings The paired-end reads in FASTQ format were also aligned to the pseudogenome which was made using the 5′ and 3′ LTRs (long terminal repeat) coordinated of HERV-K(HML-2) as described by Subramanian et al. [14]. Mapping to this pseudogenome allowed for quantification of reads aligning to HERV-host junctions.

The resulting alignments were subsequently converted to the standard BAM file format utilizing samtools with its default configurations. The expression of HERV-K-human junctions was obtained from raw reads through bedtools multicov, also employing default settings. Following this, the mapped reads were quantified and normalized using the specified formula:

$$\frac{\boldsymbol{multicov\ pseudogenome\ reads}}{\boldsymbol{alignment\ rate}\ \% * \boldsymbol{reads}}$$

A pseudogenome was constructed using the coordinates of LTRs from HERV-K(HML-2) to identify the expression of LTR-host junctions [15]. The LTR coordinates utilized for the development of the pseudogenome were detailed by Subramanian et al. The incorporation of HERVs into the human genome leads to areas that consist of both partial LTR sequences and partial human sequences, known as LTR-host junctions. Various tools were employed to analyze these datasets, including SRA-toolkit, Samtools, Bowtie, IGV, and BedTools, which is a software specifically designed to quantify the number of reads associated with each gene.

To incorporate the integration sites for the pseudogenome, partial human sequences were utilized, commencing and concluding at a defined distance of bases from the beginning and end of LTRs (the coordinates for the start and end of LTRs are provided in the attached Excel file). Consequently, a Bed file was generated by adjusting the length of reads by 66% to encompass junctions. The formula is outlined below:

chr(1,2,3, etc) LTR start-66% length LTR start+66% length

chr(1,2,3, etc) LTR end-66% length LTR end+66% length

for any LTR of HERV.

Command “bedtools getfasta” [16] was used with default settings, in order to create a fasta file including the LTR-host junction sequences that was used as the pseudogenome. As far as it concerns the alignment of pseudogenome, use the command:

“bowtie2 -t -x bowtie2/hg19 ncbi/public/sra/....fastq -S bowtie2/....sam”

Furthermore, downloading SAMtools was used for mapping the pseudogenome, via the following commands:

1. ”wget https://github.com/samtools/samtools/releases/download/1.3.1/samtools-1.3.1.tar.bz2”
2. “tar -jvxf samtools-1.3.1.tar.bz2”
3. ”cd samtools-1.3.1”
4. “make”

“.sam file” was converted to “.bam file” by means of the commands:

“samtools view -S -b bowtie2/....sam > bowtie2/....bam”

After that, files were sorted with samtools by means of the commands:

“samtools sort bowtie2/....bam -o bowtie2/..._sorted.bam”

“samtools index bowtie2/..._sorted.bam” [17].

2.3. Statistical Analysis

Data analysis was performed in IBM SPSS 24. Chromosomes were represented using frequencies. Normalized coverage of integration sites was calculated using BEDTools and custom scripts. Statistical significance between groups was assessed using independent samples t-tests and Mann-Whitney U tests, with significance set at $p < 0.05$.

 doi:10.20944/preprints202505.1844.v1

3. Results

3.1. RNASeq Datasets

Transcriptome data were examined from 11 patients with invasive ductal carcinoma breast cancer, as detailed in Table 1, alongside 6 healthy individuals, presented in Table 2.

Table 1. RNA-seq data: Instrument ILLUMINA HiSeq 2500, strategy: RNA-seq, source: Transcriptomic, Selection: cDNA, Layout: Single (breast carcinoma).

SRR Experiment	Cell_Line	Disease_State
SRR12180250	HCC1428-LTED	adenocarcinoma
SRR7509758	HCC38	ductal carcinoma
SRR7509754	HCC39	ductal carcinoma
SRR7500823	ZR75-1	ductal carcinoma
SRR10247656	HCC38	ductal carcinoma
SRR10612305	HCC38	ductal carcinoma
SRR10502311	MDA-MB-231	Triple-negative breast cancer
SRR8743332	MCF7	ductal carcinoma
SRR7509732	HCC38	ductal carcinoma
SRR7509733	HCC38	ductal carcinoma
SRR7509721	BT549	ductal carcinoma

Table 2. RNA-seq data: Instrument: Next seq 500, strategy: RNA-seq, source: Transcriptomic, Selection: PolyA, Layout: Single, (normal breast tissue).

SRR Experiment	Cell_Line	Disease_State
SRR7687788	Normal left Stroma	normal breast tissue
SRR7687782	Normal left epi	normal breast tissue
SRR7687798	Normal left Stroma	normal breast tissue
SRR7687780	Normal left Stroma	normal breast tissue
SRR7687781	Normal left epi	normal breast tissue
SRR7687797	Normal right epi	normal breast tissue

In tables 3 and 4, the raw reads and overall alignment rate from the alignment of any RNA-seq to Human genome (hg19) are presented for breast cancer patients and healty tissue respectively. Pseudogenome reads came from alignment of any RNA sequences to Pseudogenome. Normalized coverage of integration sites:

$$\frac{multicov\ pseudogenome\ reads}{alignment\ rate\ \% * reads}$$

Table 3. Raw reads and overall alignment rate came from alignment of any RNA-seq to human genome (hg19).

Breast Cancer	Treatment	Alignment Rate (%)	Raw Reads	Pseudogenome Reads	Normalized Coverage of Integration Sites
SRR8743332 ductal	Yes	93,13	26333636	160	0,0000065
SRR10502311 Triple negative	Yes	91,25	8590444	39	0,0000050
SRR10612305 ductal	Yes	91	21682035	13	0,0000007
SRR7509754 ductal	No	95,58	1087054	23	0,0000221
SRR7509758 ductal	No	96,04	1347370	17	0,0000131
SRR12180250 adenocarcinoma	Yes	91,13	13538984	67	0,0000054

 doi:10.20944/preprints202505.1844.v1

SRR7500823 ductal	Yes	85,92	13765655	12	0,0000010
SRR10247656 ductal	Yes	93,17	60339431	108	0,0000019
SRR7509721 ductal	Yes	93,2	2075305	52	0,0000269
SRR7509732 ductal	No	94,12	1464373	38	0,0000276
SRR7509733 ductal	No	94,32	1442350	47	0,0000345

Table 4. Raw Reads and overall alignment rate came from alignment of any RNA-seq to Human genome (hg19).

Health Controls	Alignment Rate (%)	Raw Reads	Pseudogenome Reads	Normalized Coverage of Integration Sites
SRR7687780	82,47	43714492	42	0,0000012
SRR7687798	80,74	43150648	44	0,0000013
SRR7687782	84,92	63226124	43	0,0000008
SRR7687788	82,18	59081485	54	0,0000011
SRR7687781	85,98	37692344	40	0,0000012
SRR7687797	85,58	46520403	57	0,0000014

3.2. Elevated HERV Expression in Breast Cancer

Mean normalized coverage of HERV integration sites was significantly higher in breast cancer tissues (M = 0.000013) than in healthy tissues (M = 0.000001, p = 0.009) (Table 5).

Table 5. Independent samples t-test for normalized coverage of integration sites between health controls and breast cancers.

Factor	Group	N	M	df	t	p-value
Normalized coverage of integration sites	Health controls	6	0,000001	10,010	-3,210	0,009
	Breast cancers	11	0,000013			

3.3. Effect of Treatment

Patients who had not received treatment exhibited significantly higher HERV expression (mean rank = 9.00) compared to treated patients (mean rank = 4.29, p = 0.023) (Table 6).

Table 6. Mann Whitney test for normalized coverage of integration sites in cases of treatment for breast.

Factor	Treatment	N	Mean Rank	U	p-value
Normalized coverage of integration sites	No	4	9,00	2	0,023
	Yes	7	4,29		

3.4. Visualization

The visualization of files was conducted using IGV, a software application that enables the observation of reads on chromosomes [17,18]. The associated IGV web application enables users to upload FASTQ files and receive visualized outcomes. IGV genome browser confirmed increased read accumulation at specific HERV loci in cancer samples versus healthy controls (figures 1-4).

 doi:10.20944/preprints202505.1844.v1

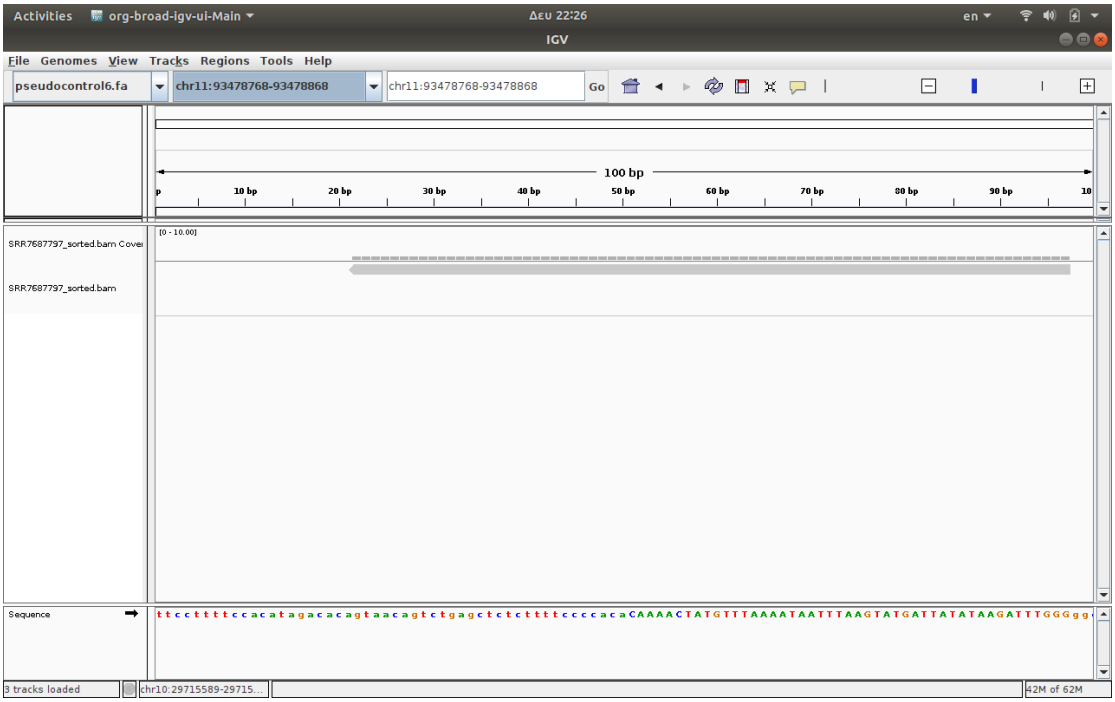

Figure 1. Visualization of a bam file of healthy breast tissue (healthy control) in chromosome 11 (chr11: 93478768-93478868) in the IGV viewer.

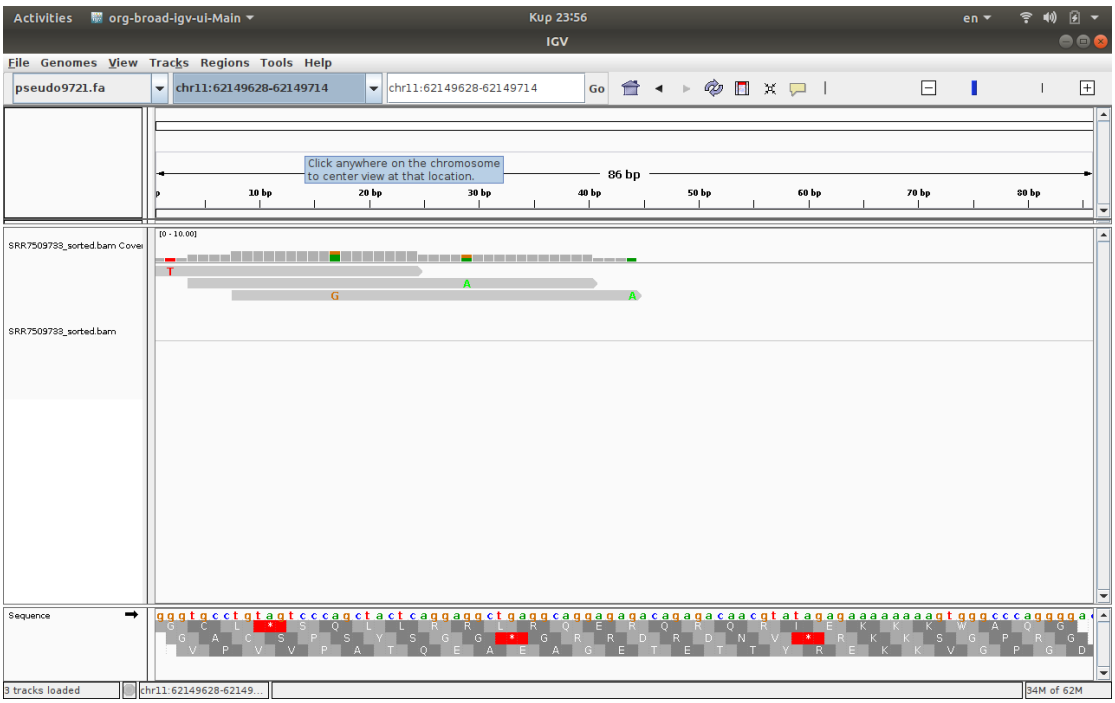

Figure 2. Visualization of a bam file of breast cancer tissue in chromosome 11 (chr11:62149628-62149714) in the IGV viewer.

 doi:10.20944/preprints202505.1844.v1

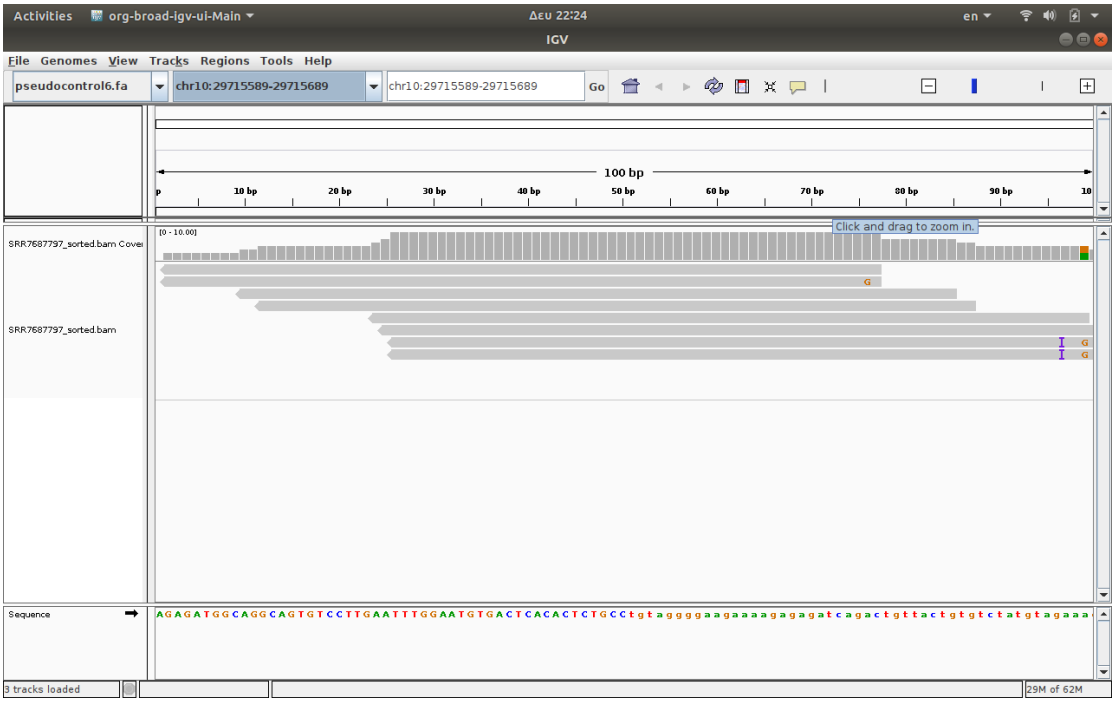

Figure 3. Visualization of a bam file of healthy breast tissue (health control) in chromosome 10 (chr10:29715589-29715689) in IGV viewer.

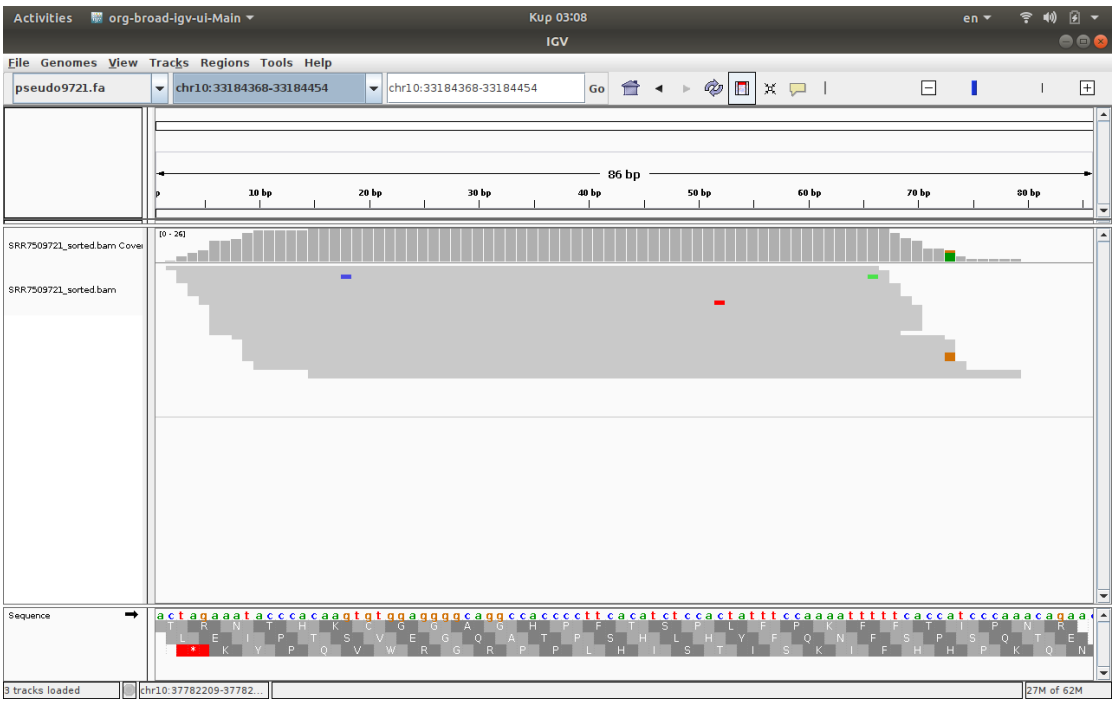

Figure 4. Visualization of a bam file of breast cancer tissue in chromosome 10 (chr10:33184368-33184454) in the IGV viewer.

4. Discussion

The present analysis investigated the expression of HERVs in breast cancer tissue compared to normal breast tissue. Furthermore, it explored the variations in normalized coverage of integration sites between breast cancer patients and healthy controls, as well as the differences between treated and untreated breast cancer cases.

The results provide compelling evidence that HERVs, specifically HERV-K (HML-2), are significantly overexpressed in breast cancer tissues compared to normal breast tissues. This

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differential expression pattern supports a growing body of literature suggesting that HERVs play a role in the etiology and progression of cancer [19–21].

Several mechanisms may explain the upregulation of HERVs in malignancies. Hypomethylation of HERV sequences and surrounding genomic regions, a common epigenetic alteration in cancer, may reactivate normally silenced proviral elements. Additionally, the tumor microenvironment, characterized by oxidative stress, cytokine production, and immune dysregulation, can further promote HERV expression [22,23]. Specifically in breast cancer, the envelope protein of HERV-K(HML-2) serves as the most effective diagnostic tool for breast cancer, as it is found in breast cancer tissues while being absent or present at significantly lower levels in normal breast tissue [24]. Also, the presence of HERV-K (HML-2) antibodies and mRNA serves as an indicator of early-stage breast cancer, with a subsequent rise suggesting the occurrence of metastasis [20,25]

The observed reduction in HERV expression among patients who received treatment is particularly noteworthy. It suggests that chemotherapy or other therapeutic regimens may suppress HERV activity, potentially by restoring epigenetic silencing mechanisms or by targeting proliferative cancer cells with elevated HERV transcription [19,26,27]. This raises important questions regarding the therapeutic targeting of HERVs, both directly and indirectly [28].

While this study offers valuable insights, limitations include the modest sample size and the reliance on public datasets without experimental validation. Further research is necessary to elucidate the functional consequences of HERV expression and its interaction with oncogenic signaling pathways.

5. Implications for Practice and Future Directions

The findings of this study have several clinical and research implications.

In clinical practice, HERV-K expression could serve as a non-invasive biomarker for early breast cancer detection [24]. The observed decrease in HERV expression following treatment suggests potential utility in tracking therapeutic efficacy. Furthermore, stratifying patients by HERV expression profiles may aid in developing tailored treatment strategies and enhancing personalized medicine approaches. The field of personalized medicine is experiencing a significant transformation due to the incorporation of multi-omics data, which primarily includes genomics, transcriptomics, proteomics, and metabolomics [29].

From a research perspective, laboratory-based validation is essential to confirm the functional role of HERVs in tumor growth and immune modulation. The immunogenic nature of HERV-derived proteins also presents opportunities for the development of novel immunotherapies, including cancer vaccines and CAR-T cell therapy [30]. Lastly, longitudinal studies monitoring HERV expression over time could provide valuable insights into disease progression, recurrence, and metastasis.

6. Conclusions

This transcriptomic analysis demonstrates significant overexpression of HERVs in breast cancer tissues, particularly in untreated patients. HERVs expression profiling may aid in diagnosis, prognosis, and treatment response monitoring in breast cancer care. With further validation, HERVs could become valuable biomarkers and therapeutic targets in oncology.

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